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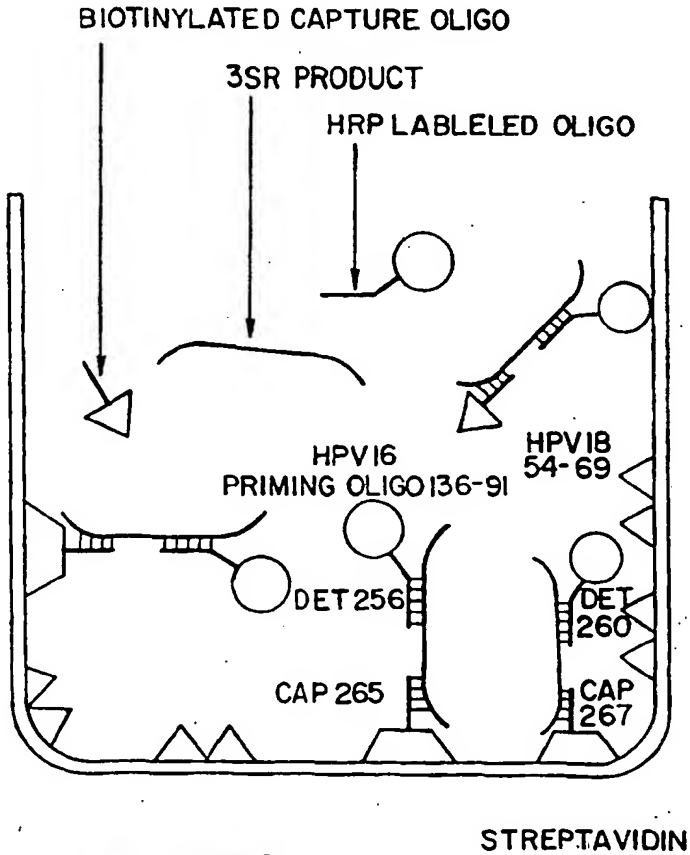
(54) Title: HUMAN PAPILLOMAVIRUS DETECTION ASSAY

(57) Abstract

A two-step nucleic acid hybridization probe assay for certain types of human papilloma virus (HPV) associated with cervical cell dysplasia and malignancy comprises a fluid phase capture hybridization step in which amplified specific gene E6/E7 messenger RNA from a biological specimen is hybridized to a biotinylated capture reagent to form a complex, attachment of the capture reagent complex to a solid phase by reaction with immobilized streptavidin, a second hybridization step in which a virus type-specific enzyme-conjugated detection probe hybridizes with the complexed amplified messenger RNA, and detection of the complexed detection probe by color or fluorophor production following a wash of the solid phase and addition of an appropriate chromogenic or fluorogenic substrate. The assay has enhanced sensitivity compared to conventional tests and is specific for actual expression of HPV oncogenes in cervical specimens, and not merely indicative of viral presence.



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HUMAN PAPILLOMAVIRUS DETECTION ASSAY

BACKGROUND OF THE INVENTION

Human papillomaviruses (HPVs) are a heterogeneous group of DNA viruses associated with a variety of proliferative lesions of the epithelium. Many of these lesions are benign such as those associated with HPV 6 and HPV 11, and are considered causative of such conditions as warts, and condylomas (see Gissman, Canc. Surv., 3: 161 (1984)). However, epidemiological and molecular studies implicate several high risk types that infect the genital tract associated with dysplasia and sometimes progress to cervical cancer (see, for example, Durst. et al., PNAS, 80: 3812 (1983)). High risk HPV types are predominately HPV 16 and HPV 18, with HPV 31, HPV 33, and HPV 35 being of lesser significance. More recently, another HPV type associated with malignancy, HPV 44, has been identified (Lorincz, U.S. Patent No. 4,849,331).

HPV of any type is generally found in extremely low numbers in biological specimens. Therefore, molecular techniques must be performed for amplifying nucleic acid viral markers from very low copy number in a specimen to detectable levels. Polymerase chain reaction (PCR) has been utilized to amplify HPV viral DNA in this manner, as disclosed in WO 90/02821, and Shibata, et al., J. Exp. Med., 167: 225 (DATE). Other applications of PCR to HPV diagnostics are Maitland, et al., May

1988. Seventh International Papillomavirus Workshop, Abstract, p. 5 and Campione-Piccardo, et al., May 1988, Seventh International Papillomavirus Workshop. One major problem with PCR amplification of HPV is that these viruses are detectable as fortuitous passengers in a significant percentage of healthy women showing no evidence of any benign of malignant pathology. Percentage estimates of such passenger presence range 10% (see U.S. Patent No. 4,983,728) to as high as 60%. Detection of HPV per se is thus of limited diagnostic value.

Many nucleic acid-based assays utilize the well-known sandwich configuration in a heterogeneous format. In this format a capture oligonucleotide is chemically conjugated to a solid support such as a microtiter well or bead, the sample is added, and the target nucleic acid having base homology to capture oligonucleotide is allowed to hybridize. After a wash (phase separation), a detection oligonucleotide hybridizes, and after a second wash to remove unhybridized detection oligonucleotide, the amount of tracer or reporter is measured, or the signal generating means produces a signal. For the details of such assays, refer to Ranki, U.S. Patent No. 4,486,539 and U.S. Patent No. 4,731,325. The basic problem with such sandwich assays is relatively low capture efficiency on the solid support, which may profoundly reduce sensitivity of the assay.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a specific assay for HPV infections associated with cervical dysplasia and cellular transformation to malignancy. In achieving this object, it is essential to first amplify to detectable levels only the messenger RNA (mRNA) expressed from oncogene regions (genes E6/E7) of HPV types implicated in malignant or pre-malignant cervical lesions. This not only restricts detection to malignant and pre-malignant HPV types, but also distinguishes actual oncogene expression from mere passenger presence of virus.

It is a further object to provide a highly sensitive assay for HPV having a high capture efficiency in the initial capture hybridization step. This is important because in situations in which the patient specimen contains very low copy number of viral mRNA, amplification may not occur to a level high enough for detection unless the assay itself is sensitive.

It is a still further aspect of the invention to provide reagents such as primer families for optimally efficient amplification, and probes which anneal to their targets under stringent conditions to give high selectivity and specificity. Finally, the invention contemplates a kit comprising these reagents, buffers, sample preparation solutions, solid supports, and reaction vessels.

In accordance with the assay of the present invention, a patient specimen suspected of containing messenger RNA encoded by at least one type of HPV associated with cervical dysplasia, malignant cells, or pre-malignant cells is

(1) subjected to nucleic acid amplification by self sustained sequence replication utilizing two primers separated by at least ten nucleotides, at least one such primer containing a transcriptional promoter,

annealing the first such primer to its complementary sequence on the target region messenger RNA, extending the 3' end of the primer by action of a strand-extending polymerase in the presence of cofactors and nucleotide triphosphates,

digesting the RNA strand of the nascent RNA/DNA duplex with an enzyme having exogenous or endogenous RNAse H activity,

annealing the second such primer to its complementary sequence on the resultant single stranded cDNA, primer extending the 3' end of the primer by action of a strand-extending polymerase,

transcribing the double stranded DNA with a transcriptase in the presence of nucleoside triphosphates, and

repeating the amplification utilizing the newly synthesized transcripts as new targets,

(2) hybridizing in solution amplified messenger RNA to a free biotinylated reagent capture probe having a sequence complementary to a first segment of the amplified RNA to form a reagent capture complex,

(3) attachment of the capture complex to a solid phase by reaction of the biotin residue of the capture probe with streptavidin bound to the surface of the solid phase,

(4) washing the bound complex to remove unbound and unreacted reagents,

(5) hybridizing a virus type-specific enzyme-conjugated detection probe having a sequence complementary to a second segment of the amplified RNA not overlapping the sequence of the first such RNA segment to form a solid phase-bound capture probe-target sequence-detection probe complex,

(6) washing the complex to remove unhybridized detection probe, and

(7) adding a fluorogenic or chromogenic enzyme substrate and reacting the conjugated enzyme to produce a detectable fluorophor or chromogen.

The present invention is also directed to certain primer families and selected probes for use in the HPV detection assay, and to kits for conveniently providing reagents to users.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: HPV 16 genome organization. Transcription proceeds clockwise from the P_{97} promotor. A_E and A_L are the polyadenylation sites for the early and late transcripts.

Figure 2: Sequence of HPV 16. The primers are indicated by underlines. Boxes indicate splice donor and acceptor sequences.

Figure 3: Sequence of HPV 18. Sequences of HPV 18 primers are indicated by underlines. Boxes indicate splice donors and acceptor sequences.

Figure 4: HPV 16 primer families. A variety of primers were tested by the ability to amplify total RNA from SiHa cells (infected with HPV 16). The reactions contained 10% DMSO and 15% sorbitol. The primers are indicated on the autoradiogram.

Figure 5: The effect of increasing the RNAse H concentration using HPV 16 primer families.

Figure 6: HPV 16 primer sensitivity. Total RNA is titrated from 1, 0.1, 0.01, 0.001 attomoles of specific E6-7 RNA isolated from SiHa RNA. p. 32. N5.

Figure 7: Primer sensitivity using cells which contain HPV 18 DNA. From right to left is 10^4 to 10 cells. p34 N4.

Figure 8: An autoradiogram slotting 3SR reaction products. A RNAse titration was performed using primers 32-54 which amplified HPV 18 RNA.

Figure 9: Autoradiogram of a 3SR reaction using primers 32-54 containing different additives. The additives (left to right) were 10% DMSO, 10% polyethylene glycol and 10% glycerol. The cross reactivity using primers 29-15 using SiHa cell using these additives were included to determine if there was any cross reactivities of the reactions.

Figure 10: Autoradiogram of a 3SR reaction comparing primers 32-54 and 69-54. The 3SR reaction using primers 69-54 contained either no additives (column 1) or 15% sorbitol (column 2). The reactions using Primers 32-54 contained 10% polyethylene glycol (column 3). From top to bottom was a titration of RNAse H, 1-3 units per reaction.

Figure 11: Co-amplification. Lane A used primers 136-73 (HPV 16), Lane B used primers 136-91 (HPV 16) amplifying 5 amol of SiHa RNA using decreasing amounts of DMSO/sorbitol mixture. Lane C from top to bottom: 136-73 (HPV 16) and 54-69 (HPV 18), 136-91 and 54-69, and 54-69 amplifying a mixture of 5 amol of SiHa cell (infected with HPV 16)

and HeLa cell (infected with HPV 18) RNA. Duplicate blots were prepared and probed with an HPV 18 specific probe (59) and an HPV 16 specific probe (98).

Figure 12: HPV 16 plate optimization. Capture 245 temperature optimum. Absorbance values using CAP245 at different temperature ranges: 30°C, 40°C, 50°C, 60°C and 70°C. Each line represents a different detectors; DET 251, DET 252, and DET 254.

Figure 13: HPV 16 plate optimization. Capture 250 temperature optimum. Absorbance values using CAP250 at different temperature ranges: 30°C, 40°C, 50°C, 60°C and 70°C. Each line represents a different detectors; DET 251, DET 252, and DET 254.

Figure 14: Detector hybridization optimum using CAP 245. Detectors were hybridized using different temperature ranges: 30°C, 40°C, 50°C, 60°C and 70°C. Each line represents different detectors: DET 98, DET 251, DET 252, and DET 254.

Figure 15: Detector hybridization optimum using CAP 250. Detectors were hybridized using different temperature ranges: 30°C, 40°C, 50°C, 60°C and 70°C. Each line represents different detectors: DET 98, DET 251, DET 252, and DET 254.

Figure 16: HPV 16 plate assay. A comparison of captures 245, 250, and 253 using DET 98, DET 251, DET 252, and DET 254. Each capture was hybridized to the 3SR product at 50°C. The detectors were hybridized at room temperature.

Figure 17: HPV 16 detector performance. A comparison of all the detector oligos for HPV 16 using CAP 250. The detector names are listed in the bottom of each figure.

Figure 18: A comparison of detector lengths using CAP 250 in the enzyme probe assay. DET 256 is a 17mer oligo and DET 257 is a 15mer oligo. The sequence was identical except that 2 bases were omitted for DET 257.

Figure 19: A comparison in absorbance values using different additives in the capture buffer. From left to right are duplicate wells using

DET 255, DET 98 and DET 256. Columns 1-6 are 3SR products using primers 96-91. Columns 7-12 are 3SR products using primer 137-91 using different detectors. The additives are indicated on the left of the absorbance values. Rows 1 and 2 are plus and minus templates using 5% polyethylene glycol. Rows 3 and 4 are plus and minus templates using 1% BSA. Rows 5 and 6 are plus and minus templates using 5% PEG, 1% BSA. Rows 7 and 8 are the standard hybridization buffer using 0.1% polyvinylpyrrolidone, 5X SSC.

Figure 20: A comparison in absorbance values using different additives in the detection buffer. From left to right using different detectors: DET 256, DET 98, and DET 255. Columns 1, 5, and 9 contained the standard hybridization buffer 30% glycerol, 0.1% PVP, 1% BSA and 5X SSC. Columns 2, 6, and 10 contained 5% PEG, 0.1% PVP, and 5X SSC as the hybridization buffer. Columns 3, 7, and 11 contained 1% BSA, 0.1% PVP and 5X SSC as the hybridization buffer. Columns 4, 8, and 12 contained 5% PEG, 1% BSA, 0.1% PVP, and 5X SSC as the hybridization buffer. Rows A and B are plus and minus templates using primers 96-91 which amplify SiHa RNA. Rows C and D is plus and minus template using primers 136-91 which amplify SiHa RNA.

Figure 21: Different primers sets which amplify HeLa RNA (HPV 18). Primers are noted on the autoradiogram.

Figure 22: Comparison of capture oligos for HPV 18 using the enzyme probe assay. The 3SR product was amplified from HeLa RNA using primer 54-69. Column 1 is substrate only. Columns 2 and 3 are plus and minus templates using capture 56. Columns 4 and 5 is plus and minus templates using capture 267. Rows indicate different detectors. Row A DET 59, Row B DET 260, Row C DET 262, Row D DET 268, Row E DET 269, and Row F DET 270.

Figure 23: Comparison of capture oligos for HPV 16 and HPV 18 using the enzyme probe assay. The 3SR product was a co-amplification from HeLa and SiHa RNA using primers 136-91 (HPV 16) and 54-69 (HPV 18).

Figure 24: HPV 16 and HPV 18 EPA. The absorbance levels of a typical specimen. HPV 16 and HPV 18 were co-amplified using primers 136-91 and 54-69. CAP 265 and CAP 267 were added and allowed to hybridize. The reaction was added to two microwells and detected using a type specific oligo DET 256 and HPV 16 and DET 260 for HPV 18.

Figure 25: Schematic of the Enzyme Probe Assay. The capture oligo hybridizes to the amplified 3SR product either HPV 16 or HPV 18. The complex is detected using HRP labeled oligonucleotide.

Figures 26 and 27: Autoradiographs of amplification products comparing yields of reaction performed at 50°C and at 42°C.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Figure 1 is a schematic drawing showing a generalized HPV 16 genome. The heavy concentric lines indicate open reading frames. Figures 2 and 3 locate the splice donor and acceptors for HPV 16 and 18 genes (indicated by boxes around the terminal two bases involved in the splice in the E6/E7 region). The portion of the HPV 16 and 18 viral genomes coding for E6/E7 polypeptides are identified in the Sequence Listing as SEQ. ID. Nos. 1 and 2 respectively. This is a significant region of the genome since the proteins encoded are thought to be involved in degradation of the p53 suppressor protein, which regulates cell growth. Loss of p53 function is associated with malignancy. Thus, expression of E6/E7 is diagnostic for cervical cancer or pre-malignant states.

In the expression of the E6/E7 region, splicing at the positions indicated in the figures occurs at substantial but unknown frequency. In designing primers for amplification of mRNA targets transcribed from this region, it is therefore important to make certain that all primer pairs lie outside the portion of the transcript from which the splice leads to excision of an mRNA fragment. Typical primers selected are illustrated in figures 2 and 3.

Since the rationale of the assay of the present invention is to detect only gene products produced in cells actually expressing genes

E6/E7, self-sustained sequence replication (3SR) is the amplification method of choice. Polymerase chain reaction amplifies DNA, and while it may detect the presence of virus with great sensitivity, it is unsuitable for detecting gene expression. The method of 3SR is fully described in Gingeras, et al., Ann. Biol. Clin., 48: 498 (1990), Guatelli, et al., PNAS, 87: 1874 (1990), and WO 90/06995. The methods described therein are followed herein except as noted, and define the procedure to be followed in the practice of the present invention. The general 3SR amplification procedure as set forth in Gingeras et al. and Guatelli et al. involves the following steps: One hundred-microliter 3SR amplification reactions contained the target RNA, 40 mM Tris-HCl at pH 8.1, 20 mM $MgCl_2$, 25 mM NaCl, 2 mM spermidine hydrochloride, 5 mM dithiothreitol, 80 μg/ml bovine serum albumin, 1 mM dATP, 1 mM dCTP, 1 mM dGTP, 1 mM dTTP, 4 mM ATP, 4 mM CTP, 4 mM GTP, 4 mM UTP, and 250 ng of each selected oligonucleotide primer. After heating at 65°C for 1 minute and cooling at 37°C for 2 minutes, 30 units of AMV reverse transcriptase, 100 units of T7 RNA polymerase, and 4 units of E. coli RNase H were added to each reaction. All reactions were incubated at 37°C for 1 hour and stopped by placing the reaction on ice.

In general, 3SR is carried out as follows on HPV specimens: samples are obtained by vaginal lavage or cervical scrape. Messenger RNA is released by treatment with chaotrophic/phenol reagents and precipitated conventionally with ethanol. A preferred one step extraction utilizes RNAzol B (Cinna/Tiotecx Laboratories, Inc.) according to the manufacturer's instructions. The RNA is then dissolved in 3SR buffer, together with nucleotide and nucleoside triphosphates, primers, enzymes, and cofactors to carry out 3SR amplification. Reagents were obtained as follows:

Primer Oligonucleotides

All oligonucleotides may be synthesized on a commercially available synthesizer such as a Milligen 8700 DNA synthesizer.

Oligonucleotides which contained a 5′ biotin may be synthesized using a biotin phosphoramidite (Glenn Research). Oligonucleotides which contain a 3′ biotin may be synthesized using control pore glass containing a protected biotin (Glenn Research). Oligonucleotides which contain a 3′ amine are conveniently synthesized using a amino-on control pore glass column (Glenn Research). Below is a list of oligonucleotides used in the development of HPV 16/18 enzyme probe assay of the present invention. All of the sequences are from left to right 5′ to 3′. The oligonucleotide primers are also listed in the Sequence Listing as SEQ. ID. Nos. 3-31.

SEQ. ID. No.	Primer Probes	
3	HPV15:	AAT TTA ATA CGA CTC ACT ATA GGG AGC TTT TCT TCA GGA CAC AGT GGC T
4	HPV19:	AAT GTT TCA GGA CCC ACA GGA GC
5	HPV20:	GAA TGT GTG TAC TGC AAG CAA CAG
6	HPV29:	ATG CAC AGA GCT GCA AAC AAC TA
7	HPV32:	CAC TTC ACT GCA AGA CAT AGA A
8	HPV48:	AAT TTA ATA CGA CTC ACT ATA GGG ATG TGT CTC CAT ACA CAG AGT C
9	HPV53:	GAA TGT GTG TAC TGCC AAG CAA CAG
10	HPV54:	AAT TTA ATA CGA CTC ACT ATA GGG AAA GGT GTC TAA GTT TTT CTG CTG G
11	HPV69:	CTG AAC ACT TCA CTG CAA GAC
12	HPV73:	CAG TTA TGC ACA GAG CTG CAA AC
13	HPV74:	GTT ATG CAC AGA GCT GCA AAC AA
14	HPV77:	CAA GCA ACA GTT ACT GCG AC
15	HPV89:	AGC AAC AGT TAC TGC GAC GT
16	HPV90:	GCA CAG AGC TGC AAA CAA CTA TA

17	HPV91:	ACA GAG CTG CAA ACA ACT ATA CA
18	HPV92:	AAT TTA ATA CGA CTC ACT ATA GGG ACT TTT CTT CAG GAC ACA GTG GCT TTT
19	HPV93:	AAT TTA ATA CGA CTC ACT ATA GGG ATT TGC TTT TCT TCA GGA CAC AGT GG
20	HPV94:	AAT TTA ATA CGA CTC ACT ATA GGG ATC TTT GCT TTT CTT CAG GAC ACA GT
21	HPV95:	AAT TTA ATA CGA CTC ACT ATA GGG ATG TCT TTG CTT TTC TTC AGG ACA CA
22	HPV96:	AAT TTA ATA CGA CTC ACT ATA GGG AGA TGT CTT TGC TTT TCT TCA GGA CA
23	HPV101:	AGA GCT GCA AAC AAC TAT ACA TG
24	HPV106:	AAT TTA ATA CGA CTC ACT ATA GGG ATT CAT GCA ATG TAG GTG TAT CTC C
25	HPV107:	AAT TTA ATA CGA CTC ACT ATA GGG ATA TTC ATG CAA TGT AGG TGT ATC T
26	HPV118:	AGC TGC AAA CAA CTA TAC ATG AT
27	HPV120:	AAT TTA ATA CGA CTC ACT ATA GGG ATG CAA TGT AGG TGT ATC TCC ATG C
28	HPV129:	AAT TTA ATA CGA CTC ACT ATA GGG AAA TGT AGG TGT ATC TCC ATG CAG
29	HPV131:	AAA CAA CTA TAC ATG ATA TAA TA

30	HPV136:	AAT TTA ATA CGA CTC ACT ATA GGG AAT GTA GGT GTA TCT CCA TGC ATG A
31	HPV137:	AAT TTA ATA CGA CTC ACT ATA GGG ATG TAG GTG TAT CTC CAT GCA TGA T

Primer selection for high level amplification is basically a directed trial and error process. To define a first set of primers a span of 400 bases (with beginning and ending sites outside the spliced region) was selected by designating the first 10-30 nucleotides at the 5′ end of the E6 gene beginning with the ATG codon and counting off 400 bases, then selecting as primers the next 10-30 bases. Note that for each pair, at least one of the primers must contain a promoter for transcription. The bacteriophage T7 RNA polymerase binding site (SEQ. ID. No. 44), AAT TTA ATA CGA CTC ACT ATA GGG A, is preferred because of its strength and specificity.

The primer pairs are tested for their amplification efficiency. To optimize, the second primer position is held stationary and the first primer is moved arbitrarily 20 bases towards the second (thereby decreasing the interprimer span, e.g. the bases between the position of the 3′ end of the first primer and the 5′ end of the second primer, by 20 bases to 380 bases). Fine tuning is accomplished by walking the primers from the best pairings by 2-5 base jumps.

Primer families. Figure 4 gives primer families that amplify the HPV 16 E6-7. All primers amplified total RNA isolated from the SiHa cell line which contain the HPV 16 transcripts. The reaction conditions include 7mM rNTPs, 1mM dNTPs, 40mM Tris pH 8.1, 30mM $MgCl_2$ 20mM KCl, 50mM dithiothreitol, 20 mM spermidine, 10% DMSO, 15% sorbitol, and 15pmol each priming oligonucleotide. After pre-warming each tube at 42°C for 5 minutes 30 units of AMV-RT, 2 units RNAse H, and 250 units of T7 RNA polymerase were added as a cocktail to each reaction. The

reaction was allowed to proceed for one hour at 42°C. A sample of the 3SR reaction was slotted onto nitrocellulose. The nitrocellulose was baked for 45 minutes and then hybridized for 45 minutes using a type specific detection oligo. An autoradiogram was generated by exposing the nitrocellulose to film for 45 minutes at -70°C. The primer family for 120 is 29 and 90. The primer family for 15 is 19, 20, 77, 53, and 89. The primer family for primer 129 is 29, 74, 73, 118, 130, and 131. The primer family for primer 136 is 91, 29, 90, 74, 73, 130, 131, and 118. The primer family for primer 137 is 29, 90, 74, 73, 131, and 118.

Figure 5 illustrates the effect of titrating the RNAse H HPV 16 primer families. The 3SR reaction conditions are identical as described in figure 4 except the DMSO and sorbitol were omitted from the reaction. Ten microliters were slotted onto nitrocellulose then baked and probed with a type specific detection oligo (HPV55). The primer family for primer 93 is 73 and 91. The optimal RNAse H needed for the reaction using these two primer pairs is between 1 and 2 units. The primer family 95 is 101 and 91. These primer sets do not appear to be sensitive to different RNAse H concentrations. A single primer set was defined for primer 92; 92-91, primer 94; 94-91, and primer 85; 85-77. The primer family for primer 96 is 73 and 91. All of these primer sets amplify optimally using between 2 and 3 units of RNAse H. The sensitivity of primers 96-73, 96-91, and 94-91 were tested using a titration of E6-7 isolated from SiHa cells. Once each primer set has been defined and optimized the sensitivity can be measured by amplifying decreasing amounts of RNA from control cells (figure 6). The 3SR reaction conditions are identical to those described in figure 4 except, using primers 96-73 the DMSO was included and the sorbitol was omitted, and using primers 94-91 only 10% sorbitol was included.

Figures 7-10 describe the primers used to amplify HPV 18 E6-7. The primer family for primer 54 is 32, 69, and 70. Primers 48 and 32 also amplify HeLa RNA. Primers 54-32 and 54-48 both require the addition of additives 10% polyethylene glycol or DMSO and sorbitol to the 3SR reaction. Primers 54-69 do not require the addition of additives for

successful amplification. Additional primer families for primer 214 is 69, 244, 214, and 70 all which require additives to the amplification reaction.

Co-amplification. Once primers have been selected for both HPV 16 and HPV 18 a co-amplification of both targets is required for clinical use. Co–amplification is required because only a single specimen is obtained. This can be done not only for HPV 16 or HPV 18, but also can be applied to a plurality of HPV types including but not limited to HPV 31, 33, and 35, as well as any other types that prove to be oncogenic. It is not practical to split a single specimen for two independent reactions. Figure 11 is a duplicate blot which is probed with a 16 and 18 type specific detection probe. Lane C demonstrates the cross reactivity of amplifying two independent targets.

Capture and Detection Probes. Because it is impractical to incubate the plate in elevated temperatures the detector should produce maximum signal at room temperature. Many times uneven temperatures across a microwell can cause differences in hybridization thereby causing variability of absorbance values. The format of the plate affects the performance of the assay. Incubating both capture and detector probes simultaneously rather than capturing the 3SR product first and detecting in a separate incubation step affects the relative OD values. There are disadvantages of co-incubation of both capture and detection probes. In high template concentration, the 3SR reaction produces very high product concentrations. When the capture is incubated to the target in one step then applied to the microwell and allowed to bind, excess target is subsequently washed away. The detection probe is then applied which only hybridizes to the capture 3SR target.

When designing capture oligonucleotide sequences, defining the hybridization temperatures is critical to the performance of the assay. Figures 12 and 13 define the optimum temperature of hybridization for HPV 16 capture oligonucleotide. The 3SR product is diluted 1:10,000 to reduce the absorbance levels thereby allowing differences of different detection probes to become more pronounced. The hybridization reaction

contain 50 µl of the diluted 3SR product in 0.1% PVP, 2X SSC, and 4 pmol capture oligonucleotide. The reaction was incubated at different temperatures ranging from room temperature to 70°C. The reaction proceeded in the microwell for 20 minutes and the well washed 3 times with 2X SSC (0.6 M NaCl, 0.06 M Na citrate pH 7.0), 0.05% Tween 20®, and 0.01% Thimersol™. The detection probe was added and incubated for 30 minutes at room temperature. The microwell was again washed 3 times with 2X SSC, 0.5% Tween 20, and 0.01% Thimersol. Substrate for the horseradish peroxidase enzyme, 3', 3', 5', 5', tetra methyl benzidine and hydrogen peroxide was added to each well and allowed to develop for 15 minutes at room temperature. The reaction was stopped by the addition of 1 M phosphoric acid and read at 450 nm.

The optimum temperature of hybridization for capture 245 is between 50°C and 60°C. The signal remains relatively constant at 70°C but thermal degradation of the RNA is a concern at this temperature. Capture 250 hybridization optimum is between 50°C and 60°C. A variety of detection probes should be tested because the optimum temperatures for hybridization of the detection probes must be empirically determined. Once the capture oligo temperature optimum has been defined, the same experiments must be repeated using different probes.

Best Mode. Figures 14 and 15 define the detector optimum. CAP 250 and CAP 245 produced the highest absorbance values when hybridizing DET 251 at room temperature. The reaction was performed as described in figure 13. The following is a list of useful detection, capture probes, and positive hybridization control probes. The detection, capture and positive hybridization control probes are also listed in the Sequence Listing as SEQ. ID. Nos. 32-43.

SEQ. ID. No.	Capture Probes:
32	CAP235: TGT ATT AAC TGT CAA AAG CCA BIOTIN
33	CAP250: TGT ATT AAC TGT CAA AAG CCA AAA AAA BIOTIN

34 CAP 253: TGT ATT AAC TGT CAA AAG CCA AAA AAA AAA A BIOTIN

35 CAP265: GTA GAG AAA CCC AGC TGT AAA AAA BIOTIN

36 CAP267: GTG CCT GCG GTG CCA GAA AAA AAA BIOTIN

SEQ. ID. No.	Detection Probes:	
37	DET59:	GAC AGT ATT GGA ACT TAC AG
38	DET98:	TTA GAA TGT GTG TAC TGC AAG NH2
39	DET255:	CAA CAG TTA CTG CGA CGT GAG NH2
40	DET256:	TTA CTG CGA CGT GAG GT NH2
41	DET260:	GTA TAT TGC AAG ACA GTA NH2

SEQ. ID. No.	Positive Hybridization Control Probes:	
42	PHC271:	TGT CTT GCA ATA TAC AAA AA BIOTIN
43	PHC272:	CTC ACG TCG CAG TAA AAA AAA BIOTIN

Figure 16 is a comparison of all the best performing capture probes using 4 different detection probes. The capture probes were hybridized to the 3SR product at the temperature optima for 30 minutes in 0.1% PVP, 2X SSC and 8 pmol capture probe. The reaction was applied to the microwell and allowed to incubate at room temperature for 20 minutes. The microwell was washed 3 times in 2X SSC, 0.05% Tween 20 and 0.01% Thimersol. The detection probe was added to the microwell and hybridized at room temperature for 30 minutes. The well was again washed 3 times and developed for 15 minutes. The reaction was stopped and read at A450. The performance of the capture probes on the plate assay could be increased by the addition of adenine residues on the end of the oligos closest to the well (data not shown). Different bases were targeted (G, C, A, and T). T was not chosen because most mRNA's are polyadenylated which would cause end hybridization. CAP 250 produces

the highest signal when amplifying SiHa cells; however, CAP 250 only can capture two of the three spliced E6 RNA's. Several other capture probes were investigated and CAP 265 captures all three E6 transcripts. Each cell line splices E6 at different rates. CAP 265 was chosen because clinical specimens may be heterogenous in splicing E6.

Once the capture probe has been defined, selecting an enzyme-conjugated detection probe is undertaken. Figure 17 is a comparison of all the detection probes for HPV 16. DET 256 produces the highest absorbance values in the present assay. Two detection probes were synthesized for illustration. The first a 17mer and the second a 15mer to define the minimum number of bases needed for efficient hybridization. The minimum length a detector oligo can be is about 17 bases (figure 18). Please note that best results are achieved when the signal enzyme is conjugated to the oligonucleotide at the 3' end.

Various additives in the capture buffer were performed with little increase in the relative absorbance in the plate assay (figure 19). When these same additives were added to the detection buffer the signal was more than doubled (figure 20). This effect appears to be related to the length of the 3SR product. The longer the product the more pronounced the effect. Primers 96-91 produce a shorter 3SR product than 136-91 (figure 20). Including additives in the detection buffer increases background levels. A titration using glycerol reduces background levels. Figure 21 is an autoradiogram of additional primer set that amplify HPV 18 using HeLa RNA. Figure 22 demonstrates the performance of HPV 18 capture probes using a variety of detection probes. Figure 23 demonstrates the absorbance values of a co-amplification and co-capture of HPV 16 and HPV 18 using type specific detection probes. Best results were achieved in co-amplification for HPV 16 and HPV 18 simultaneously utilizing primers 136-91 (HPV 16), 54-69 (HPV 18), CAP 265 (HPV 16), CAP 267 (HPV 18), and DET 256 (HPV 16), DET 60 (HPV 18) as shown in figure 24. The configuration of this assay is shown in figure 25.

The Assay Format. Utilizing the reagents described hereinabove, the assay format of the present invention was devised to optimize the signal obtainable from specimens having low viral mRNA copy number. A fluid phase capture of sample target sequence complementary to a capture prove sequence is much more efficient than adsorbing directly onto a solid phase. In fact, in a typical sandwich configuration, it is not uncommon to capture only 1-3% of total available nucleic acid in the sample. This reduces sensitivity correspondingly by two orders of magnitude.

Since it is still necessary to separate nucleic acid complexes on a solid phase, the "capture" sample must be immobilized onto the solid phase before the detection probe is added. The present assay takes advantage of the extremely high binding constant for the interaction between biotin and streptavidin. The capture oligonucleotide is biotinylated through 3′ or 5′ terminal labeling by conventional techniques. It has been empirically determined for the probes studied to date that biotinylating the capture probe at the 3′ terminus is more efficient in immobilizing the probe hybridized to sample target sequence.

The solid phase is coated with streptavidin, so that when the hybridized capture-sample sequence complex is brought into contact with it, the reaction between streptavidin and biotin takes place. The solid phase is preferably the inner surface of microtiter tray wells, but any solid phase separation system known to the art is satisfactory including but not limited to polystyrene beads, magnetic microparticles, test strips of plastic or metal, dipsticks, columns packed with a variety of materials, etc. The fluid phase capture method of the present invention is expected to give enhanced results with solid supports made of plastic because of the especially low capture efficiencies with plastic supports in conventional assays.

Any signal-generating enzyme or other reporter or tracer system capable of being conjugated covalently or electrostatically to a oligonucleotide without hindering its hybridizing to a complementary

sequence is contemplated in the present assay. Horseradish peroxidase is preferred, but alkaline phosphatase and synthetic fluorogenic and chromogenic molecule hydrolyzing enzymes may also be employed. Non-isotopic reporter/tracer systems are preferred over radioactive tracers because of environmental and stability considerations.

The kinetics of hybridization of various capture and detection probes will differ according to their thermodynamic characteristics, and some relatively insignificant amount of experimentation may be required to optimize the assay for probes of similar but not identical sequence disclosed herein for illustrative purposes.

Alternative Amplification Reaction Conditions

Figure 26 compares amplification reactions performed using the standard 3SR reaction conditions (42°C) with amplification reactions performed at an elevated temperature (50°C). The assays used the primer sets 136-91 (HPV 16) and 54-69 (HPV 18) together and separately. The standard 3SR reaction conditions were 40 mM Tris-HCl, pH 8.1; 30 mM $MgCl_2$; 20 mM KCl; 10 mM dithiothreitol; 4 mM spermidine; 15 pmole each priming oligonucleotide; 1 mM dNTP's; 7 mM rNTP's; 30 units AMV reverse transcriptase; 2 units RNAse H; and 1000 units T7 RNA polymerase. The reaction was incubated for 1 hour at 42°C. The elevated temperature reaction conditions were 40 mM Tris acetate, pH 8.1; 30 mM Mg acetate; 10 mM dithiothreitol; 100 mM potassium glutamate, pH 8.1; 1 mM dNTP's; 6 mM rNTP's; 15% sorbitol; 30 units AMV reverse transcriptase; 2 units RNAse H; and 1000 units T7 RNA polymerase. The reaction was incubated for 1 hour at 50°C.

After incubating the amplification reactions, 1/10th of the amplification products were denatured in 90 µl of 7.4% formaldehyde and 10X SSC in a 65°C water bath for 10 minutes and quick-chilled on ice for at least 1 minute. BA-85 nitrocellulose was pre-wetted with water and then with 10X SSC. The denatured amplification samples were applied to a slot blot apparatus containing the pre-wetted nitrocellulose and the samples

were drawn onto the nitrocellulose using a vacuum. The filter was then baked for 45 minutes at 80°C and hybridized with a type-specific oligonucleotide specific for HPV 18 (DET59) or HPV 16 (DET98). The hybridization solution contains 6X SSC; 10X Denhardts; 10 mM Tris, pH 7.4; 0.2 mg/ml sheared salmon sperm DNA; and 1% SDS.

Figures 26 and 27 depict a comparison of the amplification yields of reactions performed at 50°C and at 42°C. In both figures, the amplification reactions in column 1 used the HPV 16 primers 136-91, the reactions in column 2 used the HPV 18 primers 54-69, and the reactions in column 3 used a combination of the HPV 16 and HPV 18 primers 136-91 and 54-69. The target sequence was a mixture of 5 amol each of SiHa cell (infected with HPV 16) and HeLa cell (infected with HPV 18) RNA. Rows 1-4 contained sorbitol concentrations of 15%, 10%, 5% and 0% respectively; row 5 was a minus template reaction using 15% sorbitol; row 6 was blank; and rows 7-11 contained sorbitol concentrations of 15%, 10%, 5% and 0% respectively. Rows 1-5 were incubated at 50°C and rows 7-11 were incubated at 42°C. The amplification products in figure 26 were probed with DET 98 which is specific for HPV 16. The amplification products in figure 27 were probed with DET 59 which is specific for HPV 18.

Figure 26 depicts that the bands were much stronger at the 15% and 15% sorbitol levels than at the 5% or 0% levels. These results demonstrate that the increased sorbitol concentrations protect the enzymes so that the reaction can be incubated at 50°C rather than 42°C. When the sorbitol concentration was dropped below 10% the enzymes were not thermally protected and denatured at elevated temperatures, resulting in the decreased level of amplification. Figures 26 and 27 demonstrate that the elevated temperature increased the level of amplification when compared to the 42°C reaction conditions. This was particularly evident when the target sequence was co-amplified using the mixed primer set, 136-91 (HPV 16) and 54-69 (HPV 18). The estimated level of amplification using the elevated temperature was 10 fold higher than the level of amplification using the 42°C reaction conditions.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those skilled in the art without deviating from the spirit and scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Janice T. Brown

(ii) TITLE OF INVENTION:HUMAN PAPILLOMAVIRUS DETECTION ASSAY

(iii) NUMBER OF SEQUENCES:44

(iv) CORRESPONDENCE ADDRESS

(A) ADDRESSEE: Baxter Diagnostics Inc.

(B) STREET: One Baxter Parkway, Building DP-3E

(C) CITY: Deerfield

(D) STATE: Illinois

(E) COUNTRY: USA

(F) ZIP: 60015

(v)COMPUTER READABLE FORM
(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Apple Macintosh System 7.0

(D) SOFTWARE: Macintosh Text File

(vi)CURRENT APPLICATION DATA
(A) APPLICATION NUMBER: N/A

(B) FILING DATE: N/A

(C) CLASSIFICATION: N/A

(vii)PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: US 08/058,920

(B) FILING DATE: May 6, 1993

(viii)ATTORNEY/AGENT INFORMATION
(A) NAME: Mark Buonaiuto

(B) REGISTRATION NUMBER: 31,593

(C) REFERENCE/DOCKET NUMBER: BA-4448

(ix) TELECOMMUNICATION INFORMATION
(A) TELEPHONE: 708/948-2537

(B) TELEFAX: 708/948-2642

(2) INFORMATION FOR SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 570

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Papaoviridae, Human papilloma virus

(B) STRAIN: 16

(ix) FEATURE:

(A) NAME/KEY: Portion of viral genome coding for E6/E7 polypeptides.

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Seedorf, K., Krammer, G., Durst, M.,
Suhai, S., and Rowekamp, W.

(B) TITLE: Human Papillomavirus Type 16 DNA Sequence

(C) JOURNAL: Virology

(D) VOLUME: 145

(E) ISSUE:

(F) PAGES: 181-185

(G) DATE: 1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

T ATG CAC CAA AAG AGA ACT GCA ATG TTT CAG GAC CCA CAG GAG CGA 46
Met His Gln Lys Arg Thr Ala Met Phe Gln Asp Pro Gln Glu Arg
5 10 15

```
  CCC AGA AAG TTA CCA CAG TTA TGC ACA GAG CTG CAA ACA ACT
ATA   91
  Pro Arg Lys Leu Pro Gln Leu Cys Thr Glu Leu Gln Thr Thr
Ile
5                   20                  25
30

  CAT GAT ATA ATA TTA GAA TGT GTG TAC TGC AAG CAA CAG TTA
CTG 136
10  His Asp Ile Ile Leu Glu Cys Val Tyr Cys Lys Gln Gln Leu
Leu
                    35                  40
45

15  CGA CGT GAG GTA TAT GAC TTT GCT TTT CGG GAT TTA TGC ATA
GTA  181
  Arg Arg Glu Val Tyr Asp Phe Ala Phe Arg Asp Leu Cys Ile
Val
                    50                  55
20  60

  TAT AGA GAT GGG AAT CCA TAT GCT GTA TGT GAT AAA TGT TTA
AAG  226
  Tyr Arg Asp Gly Asn Pro Tyr Ala Val Cys Asp Lys Cys Leu
25  Lys
                    65                  70
75

  TTT TAT TCT AAA ATT AGT GAG TAT AGA CAT TAT TGT TAT AGT
30  TTG  271
  Phe Tyr Ser Lys Ile Ser Glu Tyr Arg His Tyr Cys Tyr Ser
Leu
                    80                  85
90
35

  TAT GGA ACA ACA TTA GAA CAG CAA TAC AAC AAA CCG TTG TGT
GAT  316
  Tyr Gly Thr Thr Leu Glu Gln Gln Tyr Asn Lys Pro Leu Cys
Asp
40                  95                  100
105

  TTG TTA ATT AGG TGT ATT AAC TGT CAA AAG CCA CTG TGT CCT
GAA  361
45  Leu Leu Ile Arg Cys Ile Asn Cys Gln Lys Pro Leu Cys Pro
Glu
                   110                  115
120

50  GAA AAG CAA AGA CAT CTG GAC AAA AAG CAA AGA TTC CAT AAT
ATA  406
  Glu Lys Gln Arg His Leu Asp Lys Lys Gln Arg Phe His Asn
Ile
                   125                  130
55  135
```

AGG GGT CGG TGG ACC GGT CGA TGT ATG TCT TGT TGC AGA TCA TCA 451
Arg Gly Arg Trp Thr Gly Arg Cys Met Ser Cys Cys Arg Ser Ser
140 145 150

AGA ACA CGT AGA GAA ACC CAG CTG TAATC ATG CAT GGA GAT ACA 495
Arg Thr Arg Arg Glu Thr Gln Leu Met His Gly Asp Thr
155 5

CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG CAA CCA GAG ACA ACT 540
Pro Thr Leu His Glu Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr
10 15 20

GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC 570
Asp Leu Tyr Cys Tyr Glu Gln Leu Asn Asp
25 30

(2) INFORMATION FOR SEQ ID NO: 2

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH:483

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Papovaviridae, Human papilloma virus

(B) STRAIN: 18

(viii) POSITION IN GENOME
(A) CHROMOSOME/SEGMENT

(ix) FEATURE:
(A) NAME/KEY: Portion of viral genome coding for E6/E7 polypeptides.

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Cole, S., and Danos, O.
(B) TITLE: Nucleotide Sequence and Comparative Analysis of the Human Papillomavirus Type 18 Genome.
(C) JOURNAL: Journal of Molecular Biology
(D) VOLUME: 193
(E) ISSUE:
(F) PAGES: 599-608
(G) DATE: 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

```
ATG GCG CGC TTT GAG GAT CCA ACA CGG CGA CCC TAC AAG CTA CCT
45
Met Ala Arg Phe Glu Asp Pro Thr Arg Arg Pro Tyr Lys Leu Pro
                5                  10                  15

GAT CTG TGC ACG GAA CTG AAC ACT TCA CTG CAA GAC ATA GAA ATA
90
Asp Leu Cys Thr Glu Leu Asn Thr Ser Leu Gln Asp Ile Glu Ile
            20                  25                  30

ACC TGT GTA TAT TGC AAG ACA GTA TTG GAA CTT ACA GAG GTA TTT
135
Thr Cys Val Tyr Cys Lys Thr Val Leu Glu Leu Thr Glu Val Phe
        35                  40                  45

GAA TTT GCA TTT AAA GAT TTA TTT GTG GTG TAT AGA GAC AGT ATA
180
Glu Phe Ala Phe Lys Asp Leu Phe Val Val Tyr Arg Asp Ser Ile
    50                  55                  60

CCG CAT GCT GCA TGC CAT AAA TGT ATA GAT TTT TAT TCT AGA ATT
225
Pro His Ala Ala Cys His Lys Cys Ile Asp Phe Tyr Ser Arg Ile
65                  70                  75

AGA GAA TTA AGA CAT TAT TCA GAC TCT GTG TAT GGA GAC ACA TTG
270
Arg Glu Leu Arg His Tyr Ser Asp Ser Val Tyr Gly Asp Thr Leu
            80                  85                  90

GAA AAA CTA ACT AAC ACT GGG TTA TAC AAT TTA TTA ATA AGG TGC
315
Glu Lys Leu Thr Asn Thr Gly Leu Tyr Asn Leu Leu Ile Arg Cys
```

95 100 105

CTG CGG TGC CAG AAA CCG TTG AAT CCA GCA GAA AAA CTT AGA CAC
360
Leu Arg Cys Gln Lys Pro Leu Asn Pro Ala Glu Lys Leu Arg His
110 115 120

CTT AAT GAA AAA CGA CGA TTT CAC AAC ATA GCT GGG CAC TAT AGA
405
Leu Asn Glu Lys Arg Arg Phe His Asn Ile Ala Gly His Tyr Arg
125 130 135

GGC CAG TGC CAT TCG TGC TGC AAC CGA GCA CGA CAG GAA CGA CTC
450
Gly Gln Cys His Ser Cys Cys Asn Arg Ala Arg Gln Glu Arg Leu
140 145 150

CAA CGA CGC AGA GAA ACA CAA GTA TAATATTAA
483 Gln Arg Arg Arg Glu Thr Gln Val
155

(2) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV15. Phage T7 RNA polymerase binding site at 5'end, followed by HPV-16/18 sequence.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

AATTTAATAC GACTCACTAT AGGGAGCTTT TCTTCAGGAC ACAGTGGCT
49

(2) INFORMATION FOR SEQ ID NO: 4

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV19.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

AATGTTTCAG GACCCACAGG AGC 23

(2) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:

(A) NAME/KEY: HPV20.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

GAATGTGTGT ACTGCAAGCA ACAG 24

(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(ix) FEATURE:
(A) NAME/KEY: HPV29.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

ATGCACAGAG CTGCAAACAA CTA 23

(2) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 22

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV32.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

CACTTCACTG CAAGACATAG AA 22

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 46

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV48.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

AATTTAATAG CACTCACTAT AGGGATGTGT CTCCATACAC AGAGTC
46

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV53.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

GAATGTGTGT ACTGCCAAGC AACAG 25

(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV54.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

AATTTAATAC GACTCACTAT AGGGAAAGGT GTCTAAGTTT TTCTGCTGG 49

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV69.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

CTGAACACTT CACTGCAAGA C 21

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV73.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

CAGTTATGCA CAGAGCTGCA AAC 23

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV74.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

GTTATGCACA GAGCTGCAAA CAA 23

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV77.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

CAAGCAACAG TTACTGCGAC 20

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV89.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

AGCAACAGTT ACTGCGACGT 20

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV90.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

GCACAGAGCT GCAAACAACT ATA 23

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV91.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17

ACAGAGCTGC AAACAACTAT ACA 23

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 51

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV92.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

AATTTAATAC GACTCACTAT AGGGACTTTT CTTCAGGACA CAGTGGCTTT T 51

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 50

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV93.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

AATTTAATAC GACTCACTAT AGGGATTTGC TTTTCTTCAG GACACAGTGG 50

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 50

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV94.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

AATTTAATAC GACTCACTAT AGGGATCTTT GCTTTTCTTC AGGACACAGT
50

(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 50

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV95.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

AATTTAATAC GACTCACTAT AGGGATGTCT TTGCTTTTCT TCAGGACACA
50

(2) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 50

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV96.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22

AATTTAATAC GACTCACTAT AGGGAGATGT CTTTGCTTTT CTTCAGGACA
50

(2) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV101.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

AGAGCTGCAA ACAACTATAC ATG 23

(2) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV106.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24

AATTTAATAC GACTCACTAT AGGGATTCAT GCAATGTAGG TGTATCTCC 49

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV107.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25

AATTTAATAC GACTCACTAT AGGGATATTC ATGCAATGTA GGTGTATCT 49

(2) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV118.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26

AGCTGCAAAC AACTATACAT GAT 23

(2) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:

(A) NAME/KEY: HPV120. Phage T7 RNA polymerase binding site at 5'end, followed by HPV-16/18 sequence.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27

AATTTAATAC GACTCACTAT AGGGATGCAA TGTAGGTGTA TCTCCATGC 49

(2) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 48

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV129.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28

AATTTAATAC GACTCACTAT AGGGAAATGT AGGTGTATCT GGATGCAT 48

(2) INFORMATION FOR SEQ ID NO: 29

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV131.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29

AAACAACTAT ACATGATATA ATA 23

(2) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV136. Phage T7 RNA polymerase binding site at 5'end, followed by HPV-16/18 sequence.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

AATTTAATAC GACTCACTAT AGGGAATGTA GGTGTATCTC CATGCATGA
49

(2) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 49

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: HPV137.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31

AATTTAATAC GACTCACTAT AGGGATGTAG GTGTATCTCC ATGCATGAT
49

(2) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: CAP245.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32

TGTATTAACT GTCAAAAGCC A 21

(2) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: CAP250.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33

TGTATTAACT GTCAAAAGCC AAAAAAA 27

(2) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: CAP253.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34

TGTATTAACT GTCAAAAGCC AAAAAAAAAA A 31

(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: CAP265.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35

GTAGAGAAAC CCAGCTGTAA AAAA 24

(2) INFORMATION FOR SEQ ID NO:36

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 24

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: CAP267.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36

GTGCCTGCGG TGCCAGAAAA AAAA 24

(2) INFORMATION FOR SEQ ID NO:37

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: DET59.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37

GACAGTATTG GAACTTACAG 20

(2) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: DET98.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38

TTAGAATGTG TGTACTGCAA G 21

(2) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: DET255.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39

CAACAGTTAC TGCGACGTGA G 21

(2) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 17

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: DET 256.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40

TTACTGCGAC GTGAGGT 17

(2) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 18

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: DET260.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41

GTATATTGCA AGACAGTA 18

(2) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: PHC271.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42

TGTCTTGCAA TATACAAAAA 20

(2) INFORMATION FOR SEQ ID NO:43

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:
(A) DESCRIPTION: Other nucleic acid, synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: DNA synthesizer

(ix) FEATURE:
(A) NAME/KEY: PHC272.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43

CTCACGTCGC AGTAAAAAAA A 21

(2) INFORMATION FOR SEQ ID NO:44

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44

AATTTAATAC GACTCACTAT AGGGA 25

I claim:

1. An assay of a patient specimen suspected of containing messenger RNA encoded by at least one type of HPV associated with cervical dysplasia, malignant cells, or pre-malignant cells comprising

(1) subjecting said specimen to nucleic acid amplification by self sustained sequence replication utilizing two primers separated by at least ten nucleotides, at least one such primer containing a transcriptional promoter,

annealing the first said primer to its complementary sequence on a target region of said messenger RNA, extending the 3′ end of said primer by action of a strand-extending polymerase in the presence of cofactors and nucleotide triphosphates,

digesting the RNA strand of the nascent RNA/DNA duplex with an enzyme RNAse H activity,

annealing the second said primer to its complementary sequence on the resultant single stranded cDNA, primer extending the 3′ end of the primer by action of a strand-extending polymerase,

transcribing the double stranded DNA with a transcriptase in the presence of nucleoside triphosphates, and

repeating the amplification utilizing the newly synthesized transcripts as new targets,

(2) hybridizing in solution amplified messenger RNA to a free biotinylated reagent capture probe have a sequence complementary to a first segment of the amplified RNA to form a reagent capture complex,

(3) attaching said capture complex to a solid phase by reaction of the biotin residues of said capture probe with streptavidin covalently bound to the surface of said phase,

(4) washing the bound capture complex to remove unbound and unreacted reagents,

(5) hybridizing a virus type-specific reporter-conjugated detection probe having a sequence complementary to a second

segment of the amplified RNA not overlapping the sequence of the first such RNA segment to form a solid phase-bound capture probe-target sequence-detection probe complex,

(6) washing the complex to remove unhybridized detection probe, and

(7) adding a fluorogenic or chromogenic enzyme substrate and reacting the conjugated enzyme to produce a detectable fluorophor or chromogen.

2. An assay for detecting HPV in a cervical specimen associated with cervical dysplasia or premalignant or malignant cells comprising

(1) amplifying target HPV messenger RNA encoding sequences contained in the viral E6/E7 region which is contained in said specimen by self sustained sequence replication,

(2) capturing said amplified messenger sequences by fluid hybridization with a biotinylated capture probe having a sequence complementary thereto,

(3) reacting said hybridized capture prove with a streptavidin coated solid phase,

(4) washing to remove unbound hybridized capture probe,

(5) hybridizing a detection probe to said target sequence,

(6) washing said solid phase, and

(7) detecting the detecting probe.

3. An assay for detecting HPV in a cervical specimen associated with cervical dysplasia or premalignant or malignant cells comprising

(1) coamplifying a plurality of oncogenic HPV type messenger RNAs contained in said specimen and having sequences encoding the respective E6/E7 genes of the HPV types or portions thereof,

(2) capturing said amplified messenger sequences by fluid hybridization with a biotinylated capture probe having a sequence complementary thereto,

(3) reacting said hybridized capture probe with a streptavidin coated solid phase,

(4) washing to remove unbound hybridized capture probe,

(5) hybridizing a detection probe to said target sequence,

(6) washing said solid phase, and

(7) detecting the detecting probe.

4. The assay of claims 1, 2, or 3 wherein said capture probes are selected from the group consisting of CAP245, CAP250, CAP253, CAP265 and CAP267.

5. The assay of claim 1 wherein the human papillomavirus-16 primers for self sustained sequence replication are selected from the group of primer pairs consisting of HPV 16: 120-29, 120-90; 15-19, 15-20, 15-77, 15-53, 15-89, 15-29; 129-29, 129-74, 129-73, 129-118, 129-130, 129-131; 136-91, 136-29, 136-90, 136-74, 136-73, 136-130; 137-29, 137-90, 137-74, 137-73, 137-118; 93-73; 93-91; 85-77; 95-101, 95-91; 96-91, 96-73; 136-131; 94-91.

6. The assay of claims 1, 2, or 3 wherein said detection probes are selected from the group consisting of DET256, DET255, DET98 and DET260.

7. Primer pairs for self sustained sequence amplification of the E6/E7 region of HPV-16 associated with cervical dysplasia or premalignant or malignant cervical cells consisting of: 15-19, 15-20, 15-77, 15-53, 15-89, 15-29; 136-91, 136-29, 136-90, 136-74, 136-73, 136-130, 136-131, 136-118; 96-91, 96-73; and 94-91.

8. Capture probes for capturing amplified RNA target sequences of the HPV E6/E7 region consisting of CAP265 and CAP267.

9. Detection probes hybridizing to the E6/E7 region of HPV consisting of enzyme-conjugated probes having the sequence of DET256, DET255, DET98 and DET260.

10. Primer pairs for self sustained sequence amplification of the E6/E7 region of HPV-18 associated with cervical dysplasia or premalignant or malignant cervical cells consisting of: 54-69, 54-70, 54-32.

11. The assay of claim 1 wherein the HPV-18 primers for self sustained sequence replication are selected from the group of primer pairs consisting of: 54-32, 54-69, 54-70; 48-32; 214-69, 214-244, 214-214, 214-70.

12. A kit for detection of HPV associated with cervical dysplasia, premalignant or malignant cervical cells comprising any of the primer pairs of claims 7 or 10, any of the capture probes of claim 8, and any of the detection probes of claim 9.

13. The assay of claim 1 wherein said nucleic acid amplification by self sustained sequence replication is performed at an elevated temperature of about 50°C in the presence of a thermal protection agent.

14. The assay of claim 2 wherein said amplifying of said target RNA is performed at an elevated temperature of about 50°C in the presence of a thermal protection agent.

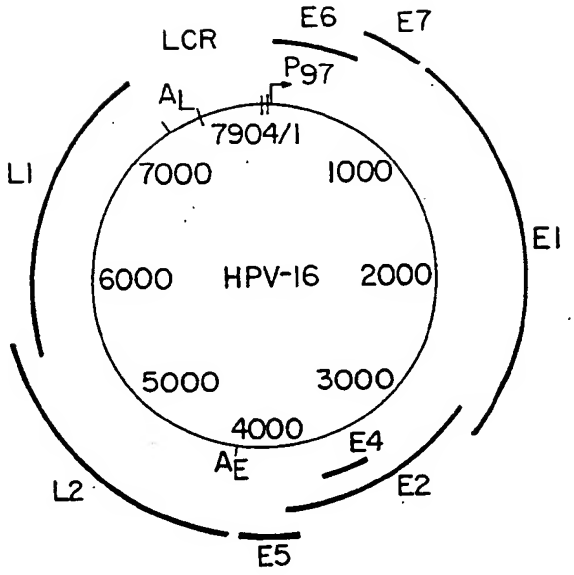
15. The assay of claim 3 wherein said coamplifying of said plurality of RNAs is performed at an elevated temperature of about 50°C in the presence of a thermal protection agent.

16. The assay of claim 1 wherein said patient sample is suspected of containing messenger RNA encoded by the E6/E7 splice region of human papillomavirus 16 or 18.

17. The assay of claim 2 wherein said viral E6/E7 region is from HPV 16 or 18.

18. The assay of claim 3 wherein said sequences encoding the E6/E7 genes are specific for the E6/E7 splice region of HPV 16 or 18.

Fig.1


LCR
E6
E7
P97
AL
7904/1
7000
1000
L1
E1
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HPV-16
2000
5000
3000
4000
E4
AE
L2
E2
E5

Fig. 2

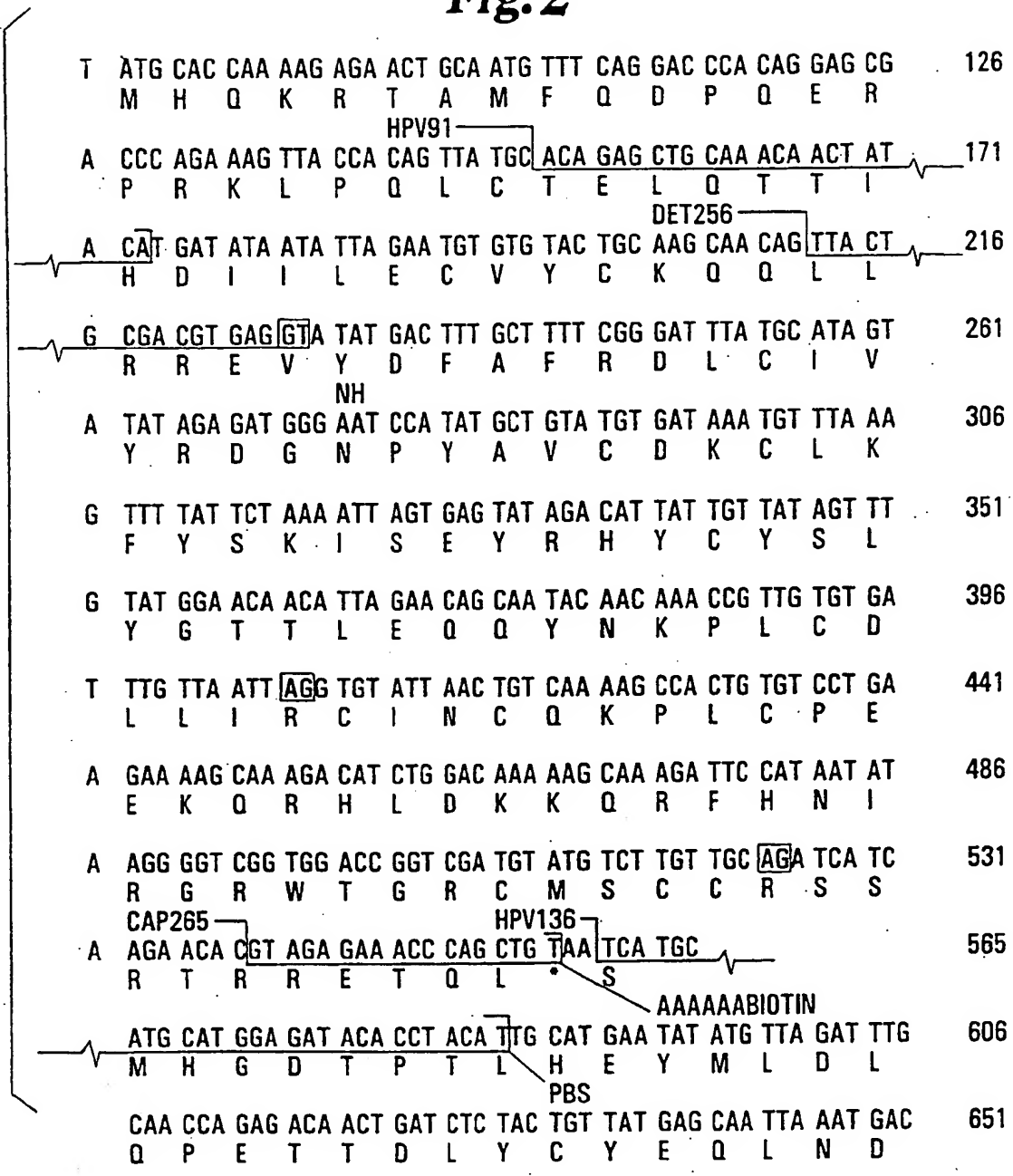

T ATG CAC CAA AAG AGA ACT GCA ATG TTT CAG GAC CCA CAG GAG CG 126
M H Q K R T A M F Q D P Q E R
HPV91
A CCC AGA AAG TTA CCA CAG TTA TGC ACA GAG CTG CAA ACA ACT AT 171
P R K L P Q L C T E L Q T T I
DET256
A CAT GAT ATA ATA TTA GAA TGT GTG TAC TGC AAG CAA CAG TTA CT 216
H D I I L E C V Y C K Q Q L L
G CGA CGT GAG GTA TAT GAC TTT GCT TTT CGG GAT TTA TGC ATA GT 261
R R E V Y D F A F R D L C I V
NH
A TAT AGA GAT GGG AAT CCA TAT GCT GTA TGT GAT AAA TGT TTA AA 306
Y R D G N P Y A V C D K C L K
G TTT TAT TCT AAA ATT AGT GAG TAT AGA CAT TAT TGT TAT AGT TT 351
F Y S K I S E Y R H Y C Y S L
G TAT GGA ACA ACA TTA GAA CAG CAA TAC AAC AAA CCG TTG TGT GA 396
Y G T T L E Q Q Y N K P L C D
T TTG TTA ATT AGG TGT ATT AAC TGT CAA AAG CCA CTG TGT CCT GA 441
L L I R C I N C Q K P L C P E
A GAA AAG CAA AGA CAT CTG GAC AAA AAG CAA AGA TTC CAT AAT AT 486
E K Q R H L D K K Q R F H N I
A AGG GGT CGG TGG ACC GGT CGA TGT ATG TCT TGT TGC AGA TCA TC 531
R G R W T G R C M S C C R S S
CAP265
HPV136
A AGA ACA CGT AGA GAA ACC CAG CTG TAA TCA TGC 565
R T R R E T Q L * S
AAAAAABIOTIN
ATG CAT GGA GAT ACA CCT ACA TTG CAT GAA TAT ATG TTA GAT TTG 606
M H G D T P T L H E Y M L D L
PBS
CAA CCA GAG ACA ACT GAT CTC TAC TGT TAT GAG CAA TTA AAT GAC 651
Q P E T T D L Y C Y E Q L N D

Fig. 3

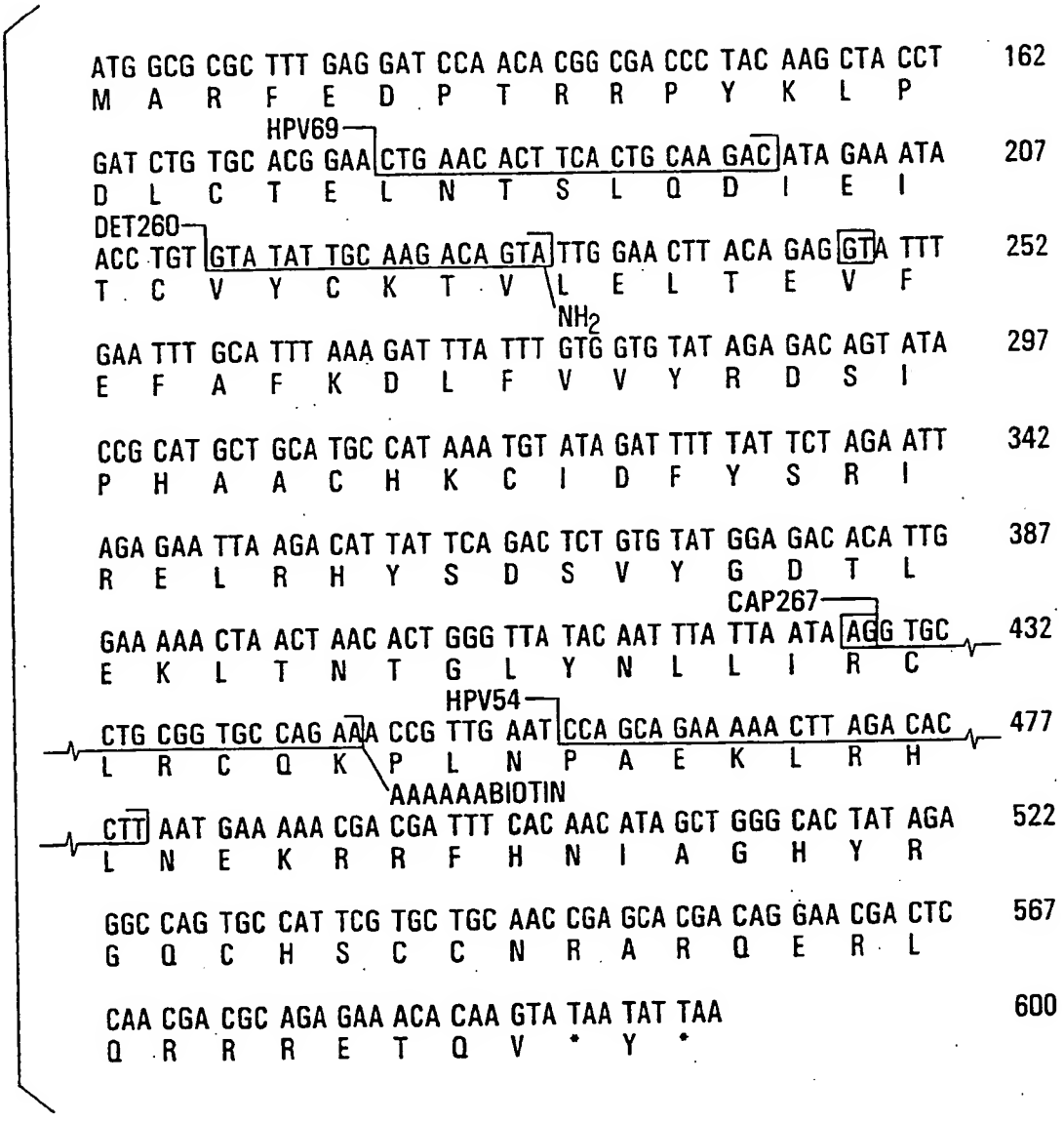
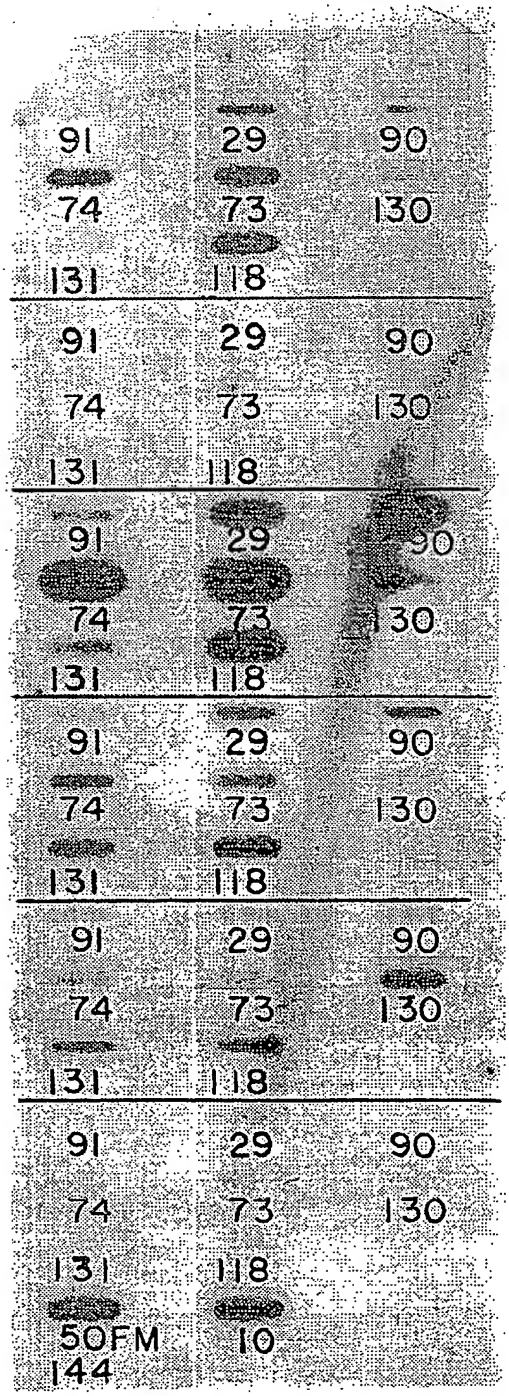

ATG GCG CGC TTT GAG GAT CCA ACA CGG CGA CCC TAC AAG CTA CCT 162
M A R F E D P T R R P Y K L P
HPV69
GAT CTG TGC ACG GAA CTG AAC ACT TCA CTG CAA GAC ATA GAA ATA 207
D L C T E L N T S L Q D I E I
DET260
ACC TGT GTA TAT TGC AAG ACA GTA TTG GAA CTT ACA GAG GTA TTT 252
T C V Y C K T V L E L T E V F
NH2
GAA TTT GCA TTT AAA GAT TTA TTT GTG GTG TAT AGA GAC AGT ATA 297
E F A F K D L F V V Y R D S I
CCG CAT GCT GCA TGC CAT AAA TGT ATA GAT TTT TAT TCT AGA ATT 342
P H A A C H K C I D F Y S R I
AGA GAA TTA AGA CAT TAT TCA GAC TCT GTG TAT GGA GAC ACA TTG 387
R E L R H Y S D S V Y G D T L
CAP267
GAA AAA CTA ACT AAC ACT GGG TTA TAC AAT TTA TTA ATA AGG TGC 432
E K L T N T G L Y N L L I R C
HPV54
CTG CGG TGC CAG AAA CCG TTG AAT CCA GCA GAA AAA CTT AGA CAC 477
L R C Q K P L N P A E K L R H
AAAAAABIOTIN
CTT AAT GAA AAA CGA CGA TTT CAC AAC ATA GCT GGG CAC TAT AGA 522
L N E K R R F H N I A G H Y R
GGC CAG TGC CAT TCG TGC TGC AAC CGA GCA CGA CAG GAA CGA CTC 567
G Q C H S C C N R A R Q E R L
CAA CGA CGC AGA GAA ACA CAA GTA TAA TAT TAA 600
Q R R R E T Q V * Y *

Fig.4

75 LS1217
2500 H2μ
T 7 2500
8.6 BUFFER
10/3 PAULSxT

10AM SiHa
6-7μg/λ
10% DMSO
15% SORB.



16100FM

134-b	136-90	136-74	136-73	HC-144
98 2PM	0.193	0.532	0.584	2.932	0.093

Fig. 5

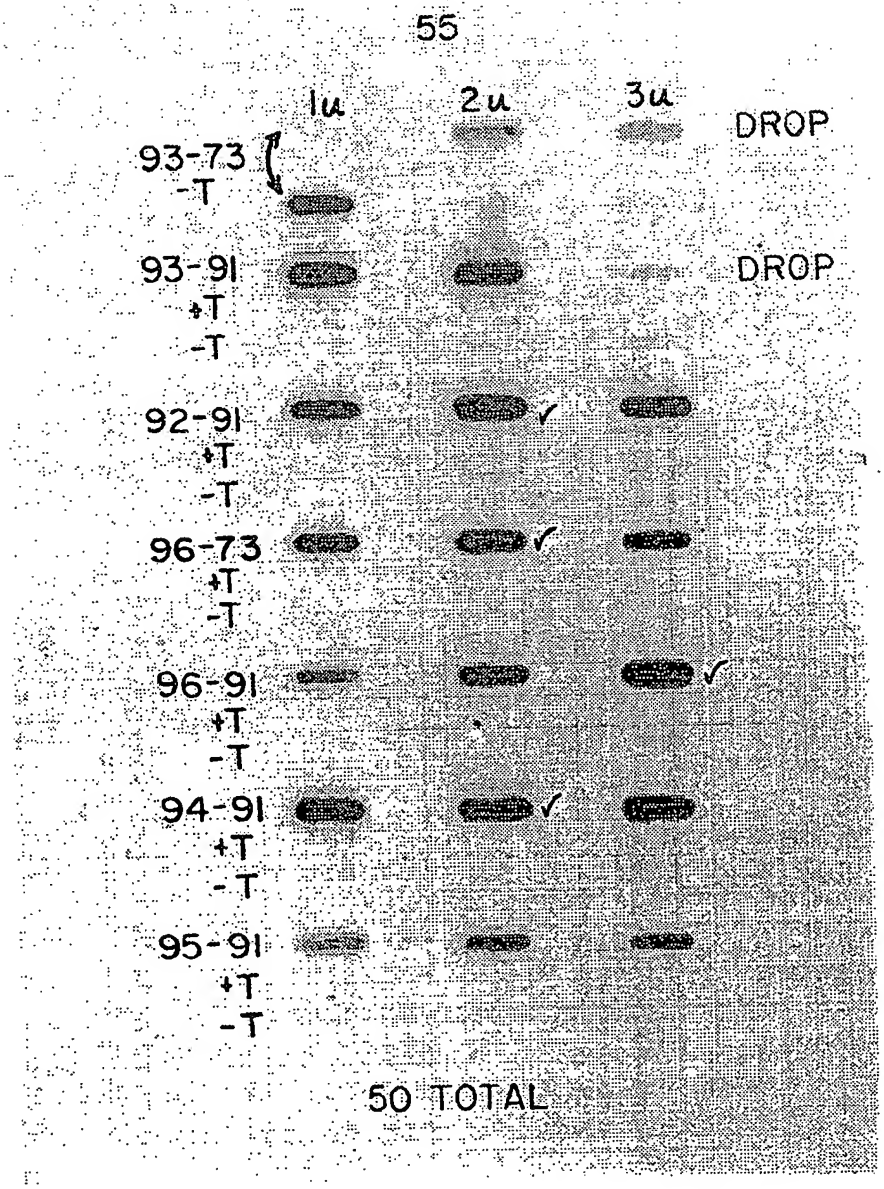

55
1u
2u
3u
DROP
93-73
-T
93-91
+T
-T
DROP
92-91
+T
-T
96-73
+T
-T
96-91
+T
-T
94-91
+T
-T
95-91
+T
-T
50 TOTAL

Fig. 6

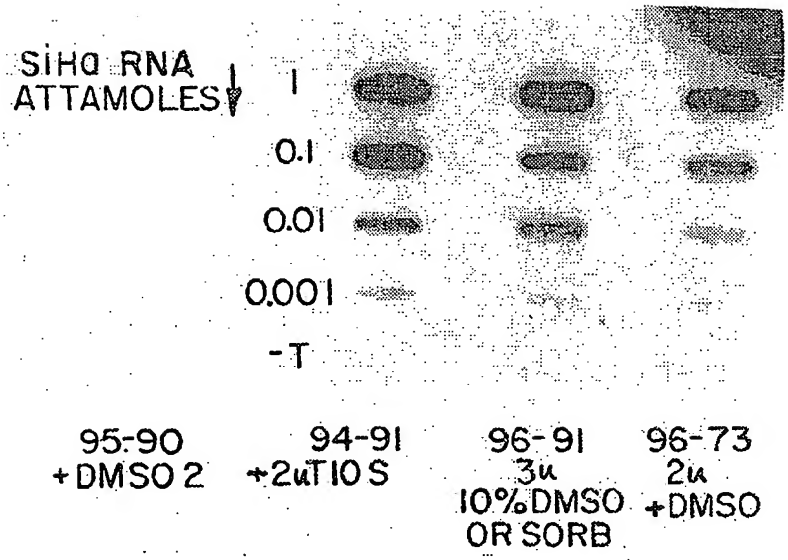

SiHa RNA
ATTAMOLES
1
0.1
0.01
0.001
-T
95-90
+DMSO 2
94-91
+2uT10S
96-91
3u
10%DMSO
OR SORB
96-73
2u
+DMSO

Fig. 7

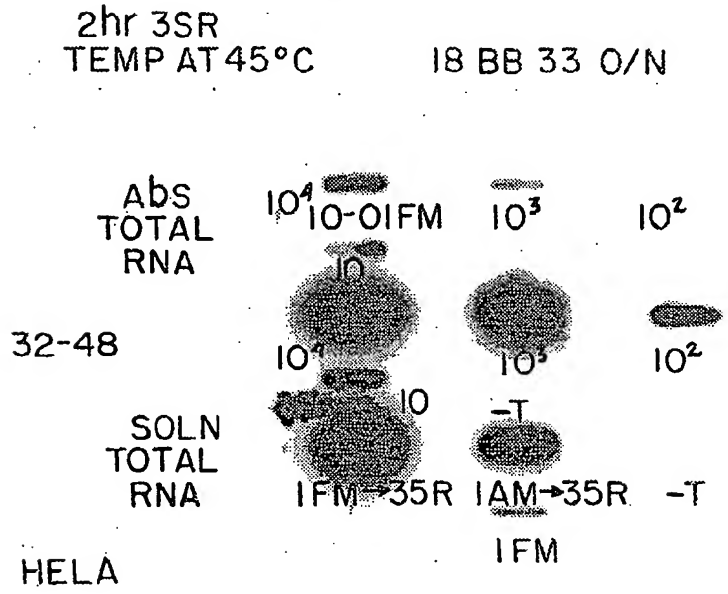

2hr 3SR
TEMP AT 45°C
18 BB 33 O/N
Abs
TOTAL
RNA
10-01FM
32-48
SOLN
TOTAL
RNA
1FM→35R
1AM→35R
-T
1FM
HELA

Fig. 8

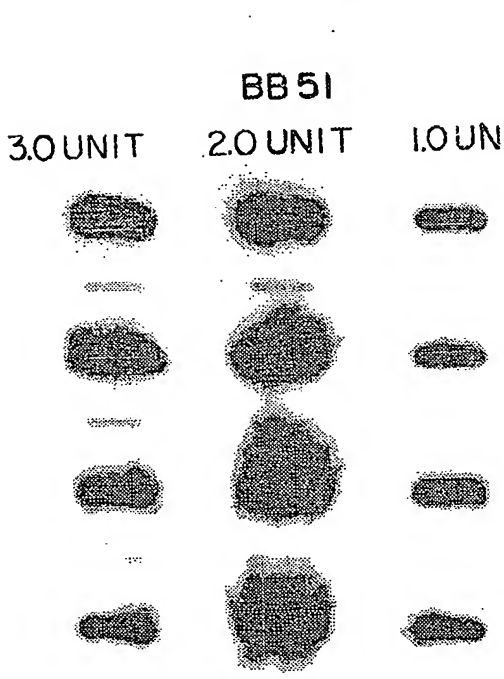

70°C 1.5 HR.
BB 51
3.0 UNIT
2.0 UNIT
1.0 UNIT
HELA
20μg
32-54 #1
-T
32-54 #1
15μ DNASe
32-54 #1
15μ DNASe
32-54 #2
-T
32-54 #2
DNASe
-T
3.0μ
2.0μ
1.0μ
32-54
SiHa
HELA PRIMERS
DNASe
3.0μ
2.0μ
1.0μ
SiHa
29-15S
DNASe

Fig. 9

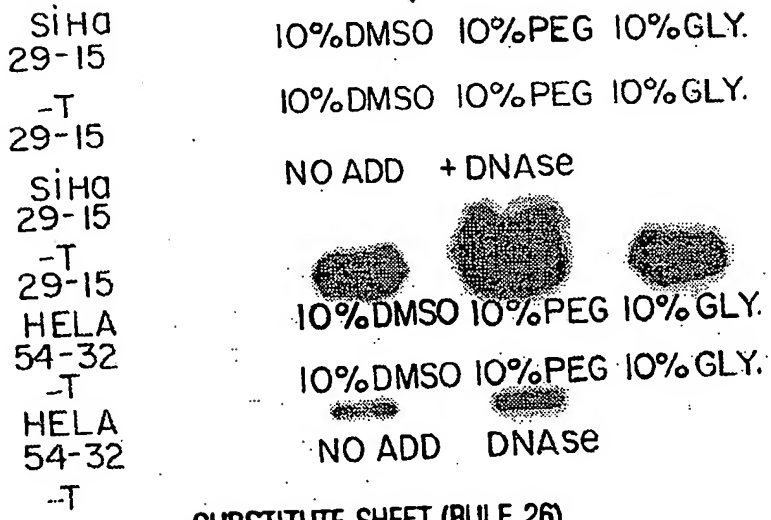

SiHa
29-15
10%DMSO 10%PEG 10%GLY.
-T
29-15
10%DMSO 10%PEG 10%GLY.
NO ADD
+DNASe
SiHa
29-15
-T
29-15
10%DMSO 10%PEG 10%GLY.
HELA
54-32
-T
10%DMSO 10%PEG 10%GLY.
HELA
54-32
-T
NO ADD
DNASe

Fig.10

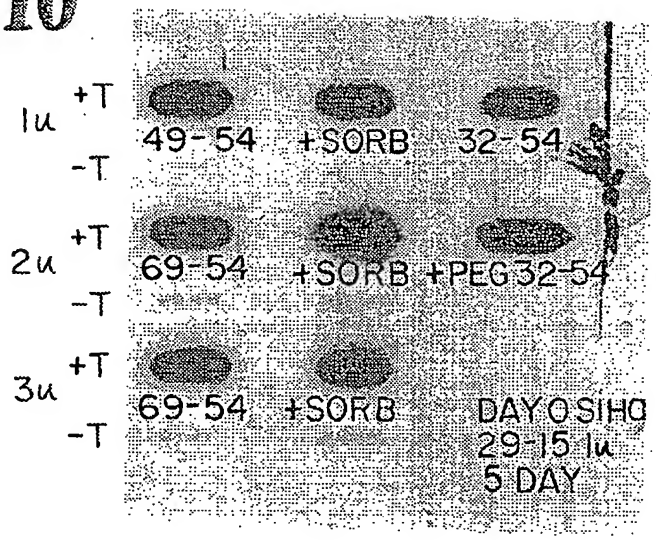

1u +T
-T
49-54
+SORB
32-54
2u +T
-T
69-54
+SORB
+PEG32-54
3u +T
-T
69-54
+SORB
DAY 0 SIHO
29-15 1u
5 DAY

Fig.11

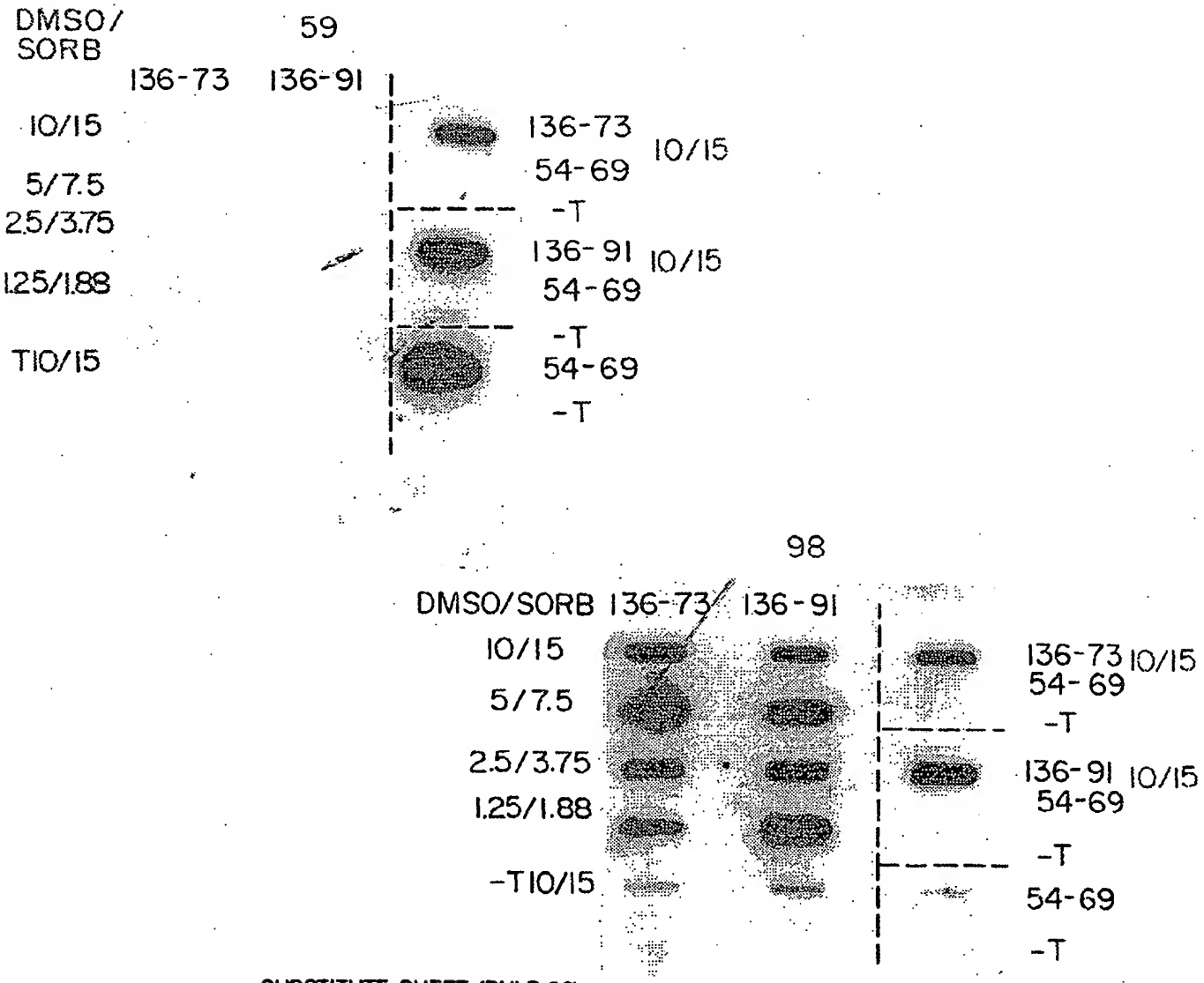

DMSO/
SORB
59
136-73
136-91
10/15
5/7.5
2.5/3.75
1.25/1.88
T10/15
136-73
10/15
54-69
-T
136-91
10/15
54-69
-T
54-69
-T
98
DMSO/SORB
136-73
136-91
10/15
5/7.5
2.5/3.75
1.25/1.88
-T10/15
136-73
10/15
54-69
-T
136-91
10/15
54-69
-T
54-69
-T

Fig. 12

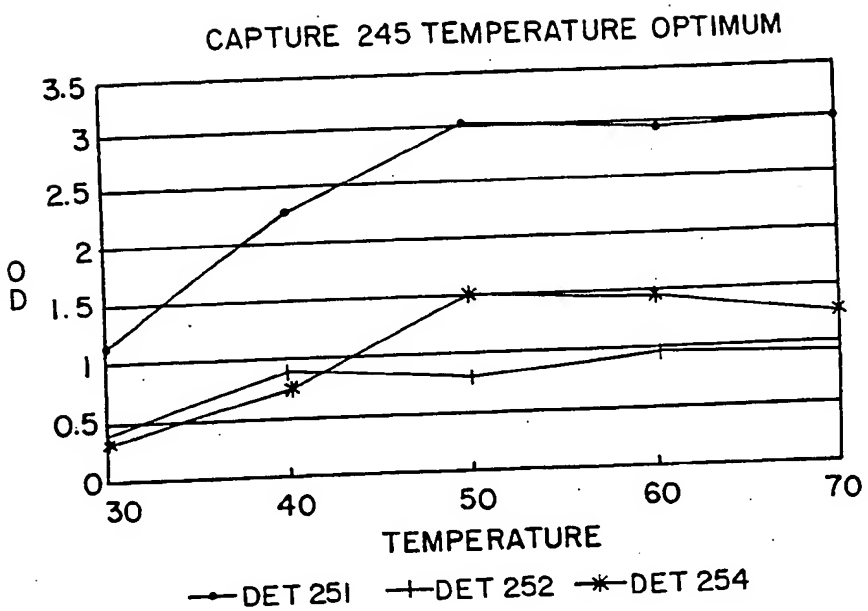

CAPTURE 245 TEMPERATURE OPTIMUM
3.5
3
2.5
2
1.5
1
0.5
0
O
D
30
40
50
60
70
TEMPERATURE
DET 251
DET 252
DET 254

Fig. 13

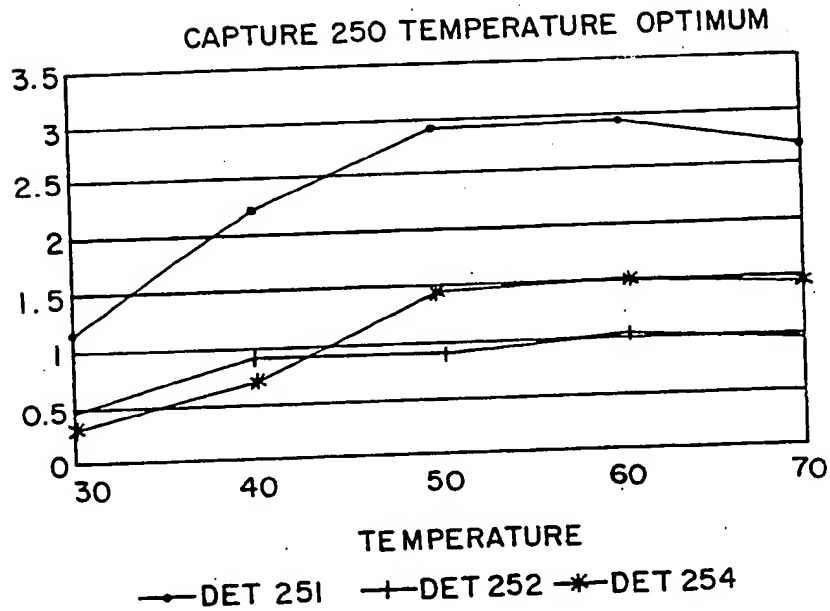

CAPTURE 250 TEMPERATURE OPTIMUM
3.5
3
2.5
2
1.5
1
0.5
0
30
40
50
60
70
TEMPERATURE
DET 251
DET 252
DET 254

Fig. 14

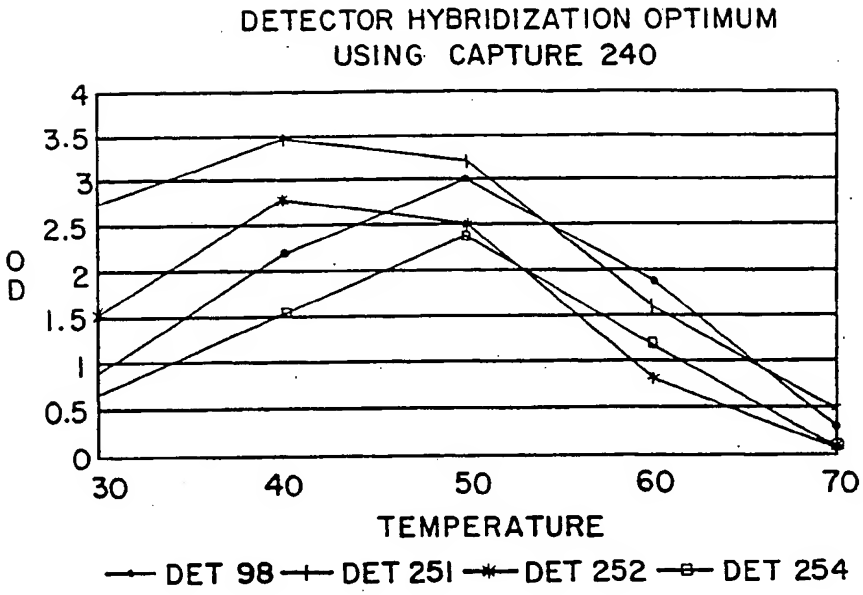

DETECTOR HYBRIDIZATION OPTIMUM
USING CAPTURE 240
OD
4
3.5
3
2.5
2
1.5
1
0.5
0
30
40
50
60
70
TEMPERATURE
DET 98
DET 251
DET 252
DET 254

Fig. 15

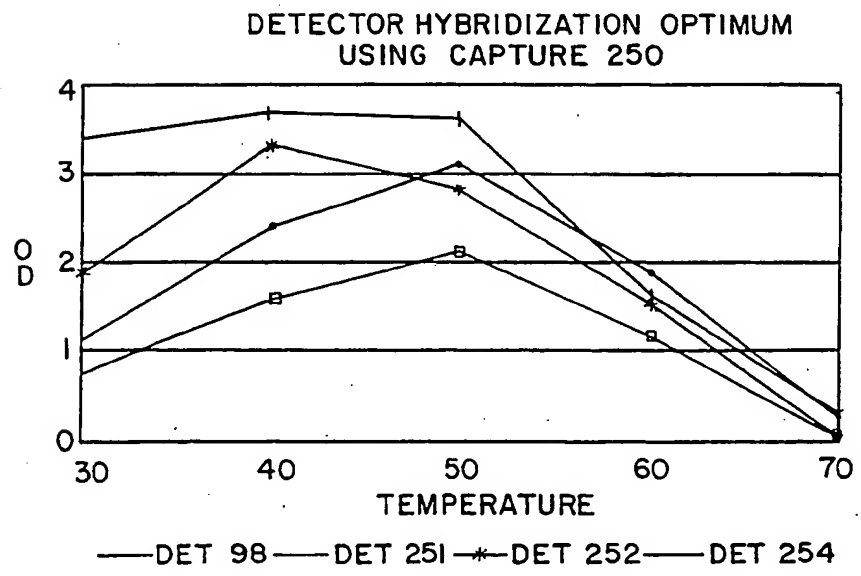

DETECTOR HYBRIDIZATION OPTIMUM
USING CAPTURE 250
OD
4
3
2
1
0
30
40
50
60
70
TEMPERATURE
DET 98
DET 251
DET 252
DET 254

Fig.16

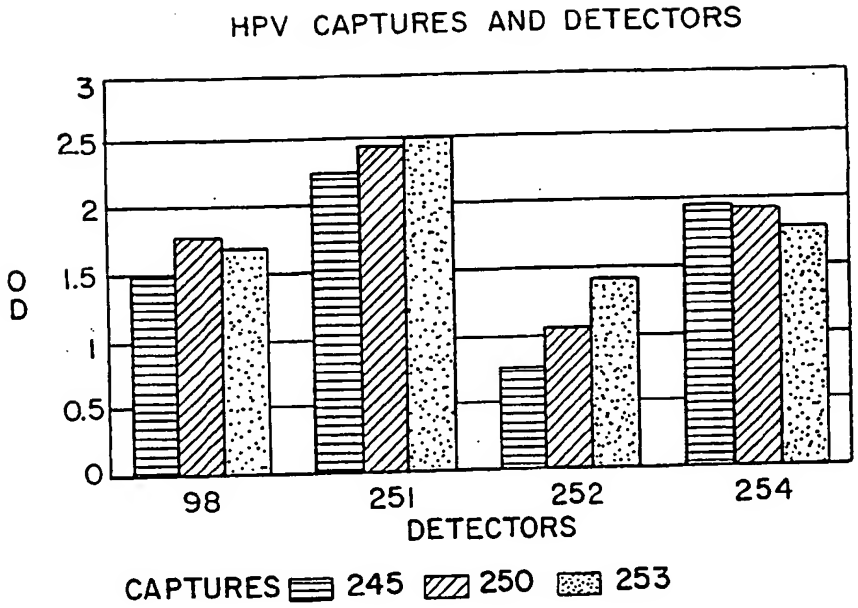

HPV CAPTURES AND DETECTORS
3
2.5
2
1.5
1
0.5
0
O
D
98
251
252
254
DETECTORS
CAPTURES 245 250 253

Fig.17

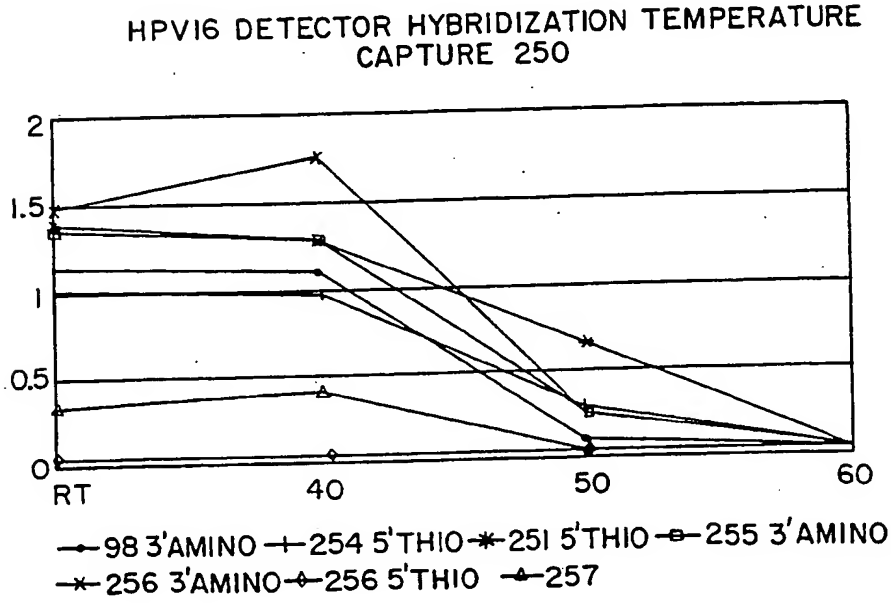

HPV16 DETECTOR HYBRIDIZATION TEMPERATURE
CAPTURE 250
2
1.5
1
0.5
0
RT
40
50
60
98 3'AMINO
254 5'THIO
251 5'THIO
255 3'AMINO
256 3'AMINO
256 5'THIO
257

Fig. 18

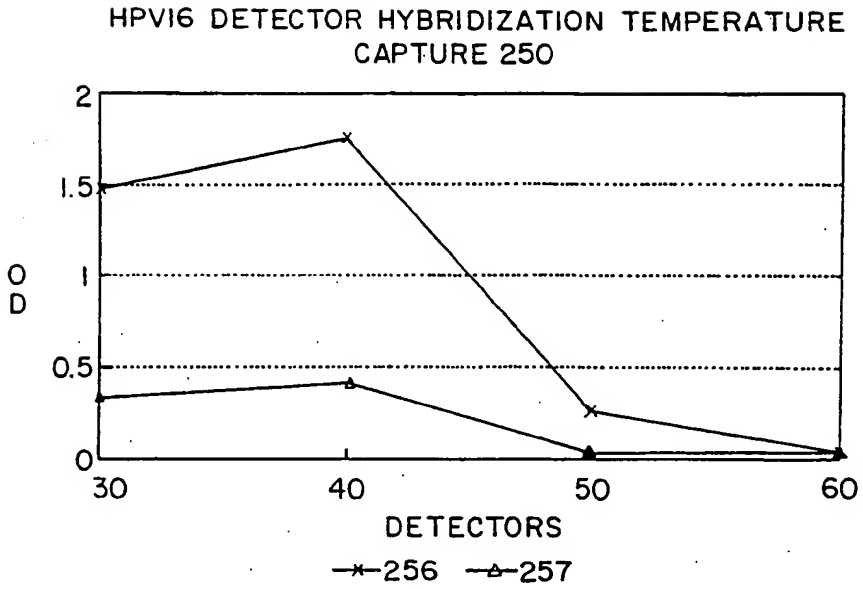

HPV16 DETECTOR HYBRIDIZATION TEMPERATURE
CAPTURE 250
2
1.5
1
0.5
0
O
D
30
40
50
60
DETECTORS
256
257

Fig.19

DESCRIPTION: ADDITIVES IN CAPTURE STEP HPV 16
PROTOCOL:
MODE: ENDPOINT AUTOMIX: OFF
WAVELENGTH: 450

WED. SEP 02 1992
4:53 PM
CALIBRATION: ON

1-6 96-91 1:200 ← OPTICAL DENSITY → 6-12 137-91

		1	2	3	4	5	6	7	8	9	10	11	12
		255-3NH2			98		256		255		98		256
PEG	A	0.477	0.667	0.450	0.535	0.242	0.316	2.205	2.848	1.004	0.883	2.230	1.998
5% -T	B	0.036	0.033	0.032	0.032	0.038	0.038	0.036	0.039	0.035	0.036	0.037	0.037
1% BSA	C	0.418	0.500	0.240	0.349	0.155	0.128	2.709	2.003	0.839	0.551	2.051	1.932
-T	D	0.030	0.032	0.036	0.034	0.037	0.034	0.036	0.030	0.034	0.034	0.037	0.039
5%P,E 1% BSA	E	0.742	0.625	0.418	0.450	0.183	0.296	2.747	2.722	0.822	0.738	2.051	1.954
-T	F	0.046	0.031	0.034	0.032	0.034	0.032	0.032	0.028	0.034	0.033	0.034	0.035
~~0~~	G	0.946	1.133	0.980	0.893	0.569	0.597	2.372	2.503	0.612	0.677	1.855	1.928
0.1% PVP 5 -T	H	0.036	0.035	0.034	0.030	0.030	0.034	0.032	0.031	0.032	0.032	0.033	0.044

ALL 250 AT 55°C 30' ALL IN 0.1% PVP, 5x5CC
ALL IN 0.1% PVP, 5x5CC
DET AT RT IN GLYCEROL BUFFER
DUPLICATE WELLS (-T= 96-91 OR 137-91 -T 3 SRrxn)

Fig. 20

DESCRIPTION: DETECTOR HYB OPTINIZATION
PROTOCOL:
MODE: ENDPOINT AUTOMIX: ON
WAVELENGTH: 450

THU SEP 03 1992
11:33 AM

CALIBRATION: ON

1:200	← 256 →				← 98 →				← 255 →			
Final	1	2	3	4	5	6	7	8	9	10	11	12
	0	5%P	1%B	5P/1BS	0	5%P	1%B	5P/1B	0	5%P	1%B	5P/1B
96-91A	0.234	0.865	0.358	0.676	0.234	0.425	0.307	0.449	0.507	1.742	1.670	2.060
-T B	0.040	0.293	0.095	0.278	0.043	0.041	0.280	0.135	0.069	1.874	1.404	2.020
C	0.545	1.269	0.747	1.313	0.266	0.586	0.344	0.523	0.632	1.547	1.396	1.908
-T D	0.038	0.429	0.128	0.359	0.051	0.042	0.042	0.052	0.039	1.474	1.123	1.359
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

STOP AT 2" CAPTURE 250, 55', 30', 0.1% PVP, 5×55C
PLATE 20'
DET 0- 30% GLYCEROL, 0.1% PVP, 1% BSA, 5×55C
5%P- 5% PEG, 0.1%PVP, 1% BSA, 5×55C
1%B- 1% BSA, 0.1% PVP, 5×55C
5P/1B- 5% PEG, 1% BSA, 0.1% PVP, 5×55C

Fig. 21

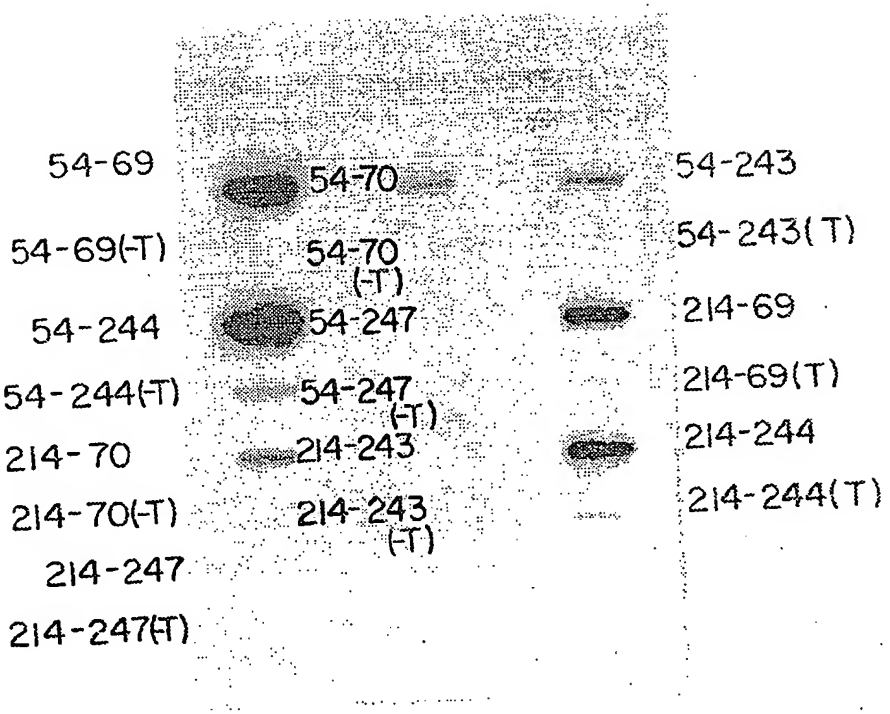

54-69
54-70
54-243
54-69(-T)
54-70 (-T)
54-243(T)
54-244
54-247
214-69
54-244(-T)
54-247 (-T)
214-69(T)
214-70
214-243
214-244
214-70(-T)
214-243 (-T)
214-244(T)
214-247
214-247(-T)

Fig. 22

RAW DATA

DATA FILE: DATA0112.001
DESCRIPTION: HPV 18 CAPTURE AND DETECTOR SELECTION
PROTOCOL:
MODE: ENDPOINT AUTOMIX: ON
WAVELENGTH: 450

TUES. JAN 12 1993
7:46 PM
CALIBRATION: ON

OPTICAL DENSITY

56 (2 − 3) 267 (4 − 5)

		1	2	3	4	5	6	7	8	9	10	11	12
59	A	0.038	0.988	0.087	1.762	0.067	0.036	0.000	0.000	0.000	0.000	0.000	0.000
260	B	0.033	1.129	0.033	2.621	0.037	0.031	0.000	0.000	0.000	0.000	0.000	0.000
262	C	0.034	0.712	0.036	2.153	0.037	0.031	0.000	0.000	0.000	0.000	0.000	0.000
268	D	0.037	0.919	0.037	2.311	0.038	0.037	0.000	0.000	0.000	0.000	0.000	0.000
269	E	0.027	0.727	0.036	1.718	0.040	0.034	0.000	0.000	0.000	0.000	0.000	0.000
270	F	0.026	0.237	0.038	0.662	0.040	0.030	0.000	0.000	0.000	0.000	0.000	0.000
	G	0.034	0.037	0.036	0.040	0.034	0.033	0.000	0.000	0.000	0.000	0.000	0.000
	H	0.029	0.120	0.038	0.039	0.038	0.034	0.000	0.000	0.000	0.000	0.000	0.000

Fig. 23

RAW DATA

DATA FILE: DATA0114.001
DESCRIPTION: HPV 16 AND 18 PLATE
PROTOCOL:
MODE: ENDPOINT
WAVELENGTH: 450

AUTOMIX: ON

THU. JAN 14 1993
5:49 PM

CALIBRATION: ON

CAPTURES

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
	BLANK	267/250	-T			18 267	-T	16 250		-T		
A	0.038	0.179	0.208	0.035	0.035	0.041	0.041	0.365	0.368	0.046	98	
B		0.722	0.589	0.048	0.037	0.040	0.037	1.179	1.274	0.095	255	
C		0.454	0.408	0.036	0.041	0.049	0.040	0.778	0.754	0.059	256	
D		2.367	2.429	0.035	2.619	2.626	0.038	0.039	0.043	0.040	260	
E		2.607	2.593	0.035	2.724	2.695	0.039	0.527	0.524	0.038	98, 260	
F		2.842	2.742	0.047	2.729	2.773	0.040	1.427	1.537	0.174	255, 260	
G		2.781	2.799	0.043	2.894	2.804	0.097	1.034	1.054	0.125	256, 260	
H		0.041	0.042	0.038	0.042	0.042	0.044	0.039	0.056	0.043		

DETECTORS

Fig. 24

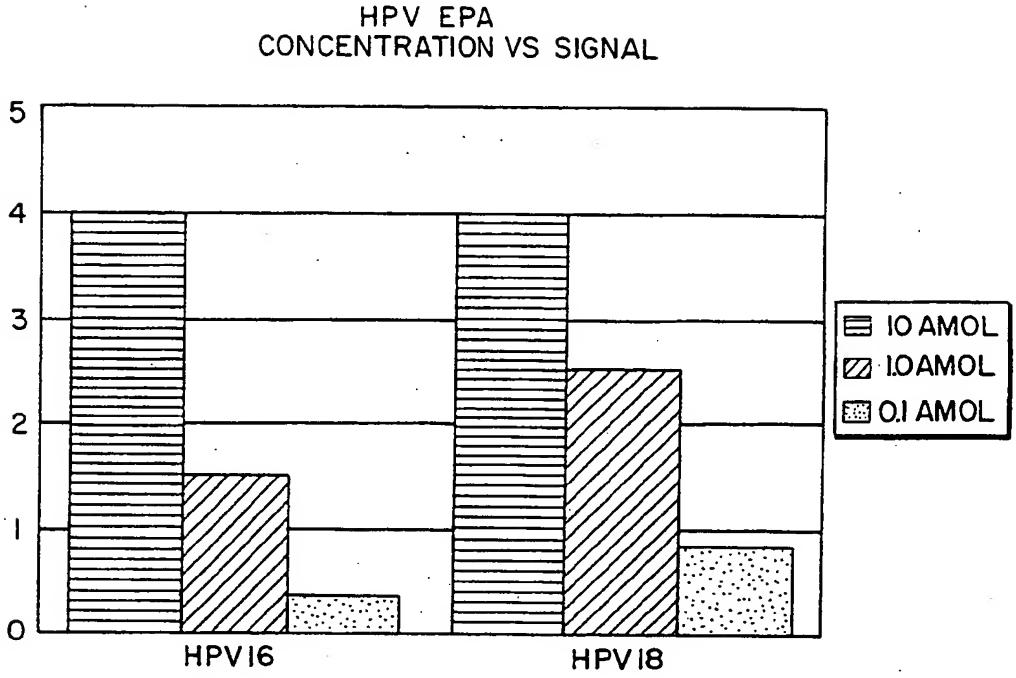

HPV EPA
CONCENTRATION VS SIGNAL
5
4
3
2
1
0
HPV16
HPV18
10 AMOL
1.0 AMOL
0.1 AMOL

Fig. 25

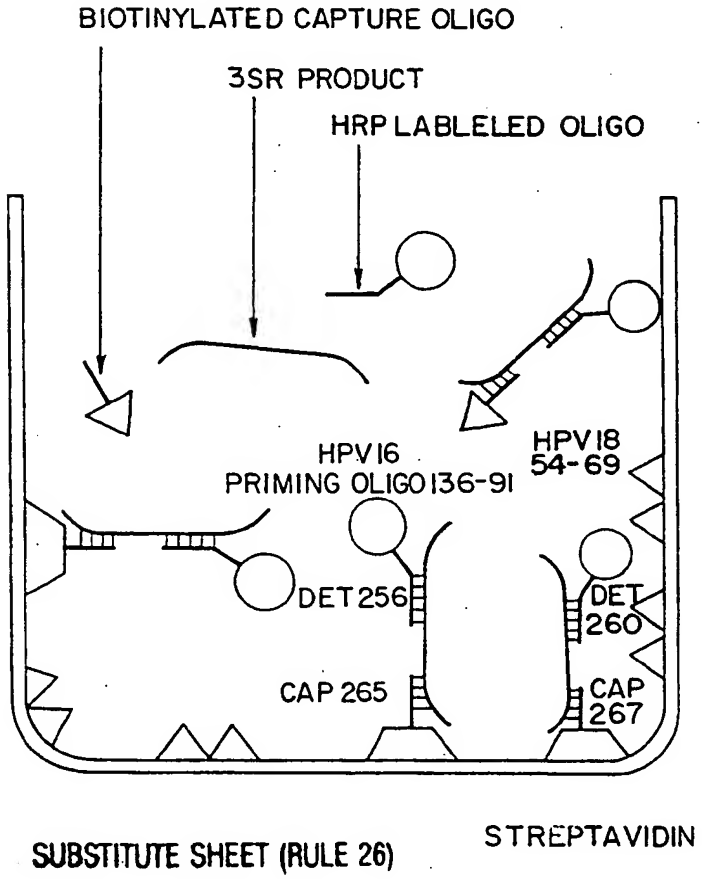

BIOTINYLATED CAPTURE OLIGO
3SR PRODUCT
HRP LABLELED OLIGO
HPV16
PRIMING OLIGO 136-91
HPV18
54-69
DET 256
DET
260
CAP 265
CAP
267
STREPTAVIDIN

Fig. 26

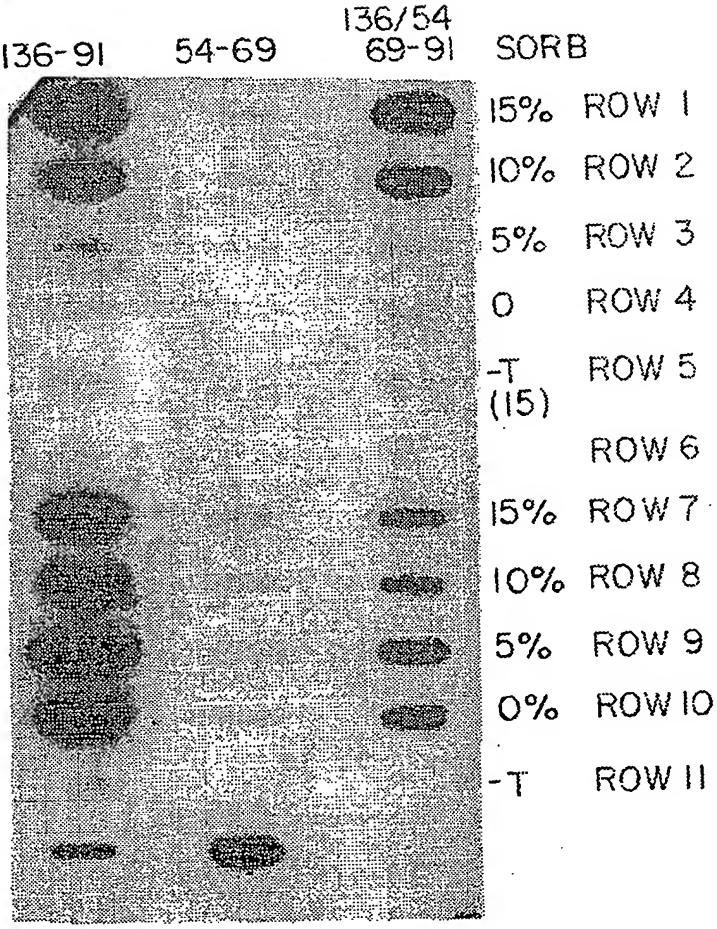
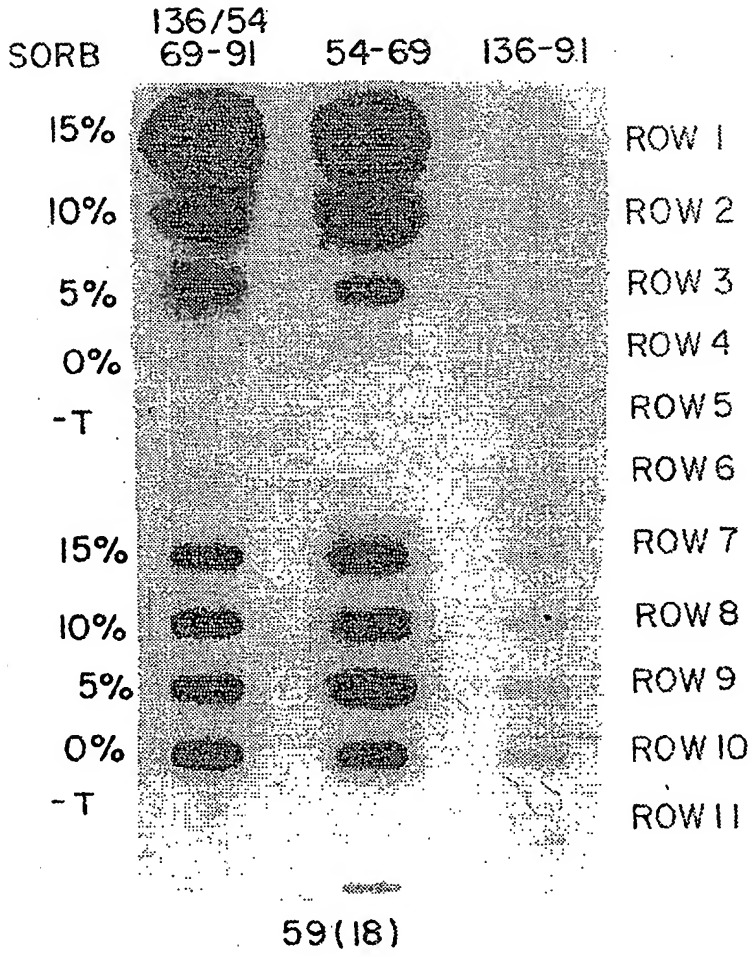

136-91
54-69
136/54
69-91
SORB
15% ROW 1
10% ROW 2
5% ROW 3
0 ROW 4
-T (15) ROW 5
ROW 6
15% ROW 7
10% ROW 8
5% ROW 9
0% ROW 10
-T ROW 11

Fig. 27


136/54
69-91
SORB
54-69
136-91
15%
10%
5%
0%
-T
15%
10%
5%
0%
-T
ROW 1
ROW 2
ROW 3
ROW 4
ROW 5
ROW 6
ROW 7
ROW 8
ROW 9
ROW 10
ROW 11
59(18)

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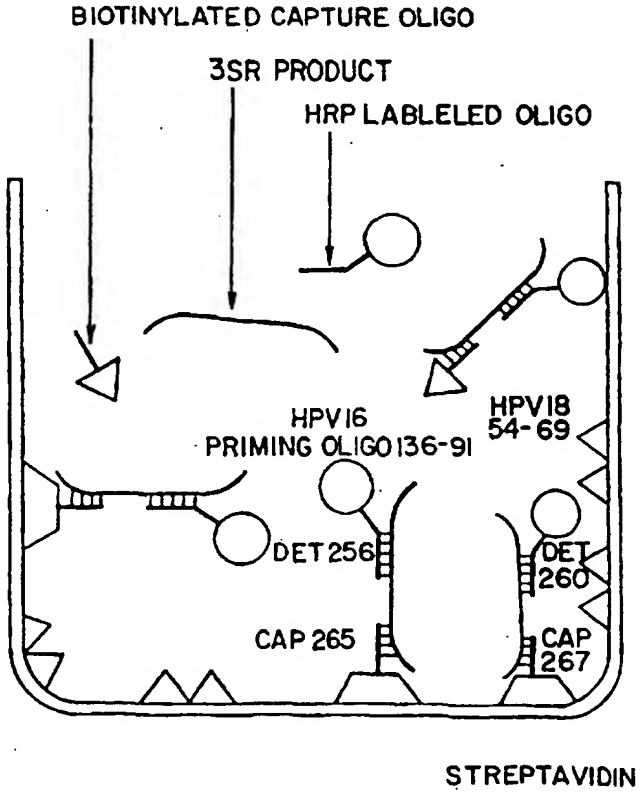
(57) Abstract

A two-step nucleic acid hybridization probe assay for certain types of human papilloma virus (HPV) associated with cervical cell dysplasia and malignancy comprises a fluid phase capture hybridization step in which amplified specific gene E6/E7 messenger RNA from a biological specimen is hybridized to a biotinylated capture reagent to form a complex, attachment of the capture reagent complex to a solid phase by reaction with immobilized streptavidin, a second hybridization step in which a virus type-specific enzyme-conjugated detection probe hybridizes with the complexed amplified messenger RNA, and detection of the complexed detection probe by color or fluorophor production following a wash of the solid phase and addition of an appropriate chromogenic or fluorogenic substrate. The assay has enhanced sensitivity compared to conventional tests and is specific for actual expression of HPV oncogenes in cervical specimens, and not merely indicative of viral presence.



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KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
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ML	Mali
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SK	Slovakia
SN	Senegal
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TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

INTERNATIONAL SEARCH REPORT

Internationa' plication No
PCT/US 94/05085

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12Q1/70 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ICAA, PROGRAM ABSTRACT, vol.31, no.0, 1991 page 334 J.T. BROWN AND E. W. RADANY 'Development of a rapid assay for the detection of HPV transforming gene expression'	1-3, 16-18
Y	31st Interscience conference on antimicrobial agents and chemotherapy, Chicago, Illinois, USA, Sept. 29-Okt. 2, 1991; abstract no. 1411 see abstract	9-12
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
30 November 1994	14. 12. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	J. CELL. BIOCHEM. SUPPL. VOL.111, NO. 5 (PART 2),, November 1990, ROCKEFELLER UNIV. PRESS, N.Y., US; page 56A J.T. BROWN ET AL. 'Isothermal enzymatic amplification of HPV RNA using the 3SR reaction' 30th Annual Meeting of the American Society for Cell Biology, 9-13 December 1990, San Diego, California, US; abstract no. 293 see abstract	1-18
Y	--- EP,A,0 420 260 (F. HOFFMANN-LA-ROCHE & CO.) 3 April 1991 see page 3, line 53 - page 4, line 34 see page 6, line 1 - page 8, line 39; claims 1-52	1-18
Y	--- EP,A,0 425 217 (CIBA CORNING DIAGNOSTICS CORP.) 2 May 1991 see claims 1-17	1-18
Y	--- WO,A,91 08312 (GENE-TRAK SYSTEMS) 13 June 1991 see page 7, line 1 - page 30, line 30; claims 1-38	1-18
Y	--- WO,A,91 19812 (BIO MERIEUX) 26 December 1991 see page 1, line 1 - page 13, line 34; claims 1-64 see examples 14-16	1-18
Y	--- WO,A,92 14847 (ORGENICS INTERNATIONAL) 3 September 1992 see page 1, line 1 - page 11, line 1; claims 1-40	5,7,10, 11
Y	--- WO,A,92 01815 (EMERY, BAVIN, WALKER) 6 February 1992 see page 1, line 1 - page 8, line 7; claims 1-10	5,7,10, 11
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US 94/05085

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 373 352 (BEHRINGWERKE) 20 June 1990 see page 4, line 21 - page 6, line 28; claims 1-7	5,7,10, 11
P,X	WO,A,93 24658 (GEN TRAK INC.) 9 December 1993 see page 9, line 15 - page 28, line 19; claims 1-26; figure 1	1-3, 16-18
P,Y	DIAGNOSTIC MOLECULAR BIOLOGY: PRINCIPLES AND APPLICATIONS, PERSING ET AL. (ED.), 0(0), 13 May 1993, XXII+641P. AMERICAN SOCIETY FOR MICROBIOLOGY (ASM): WASHINGTON, DC,US; pages 414 - 419 J.T. BROWN AND A.T. WORTMAN 'Rapid amplification of human Papillomavirus type 16 and 18 E6 and E7 mRNA by 3SR' see page 414, line 1 - page 418, line 40; tables 1,2	1-18

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US 94/05085

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